Discussion

Acute oral administration of DIMP to female mink resulted in an LD_{50} determination somewhat higher than that reported for rats (Kinkead et al., 1971; Dacre and Hart, 1977) and mice (Dacre and Hart, 1977) but less than that reported for Mallard ducks and for Bobwhite quail as previously stated in this report. These data suggest an intermediate sensitivity for mink with respect to acute DIMP poisoning.

The clinical signs of acute oral toxicity of mink dosed with DIMP were consistent with those reported for Mallards, and for Bobwhite quail, as general depressive effects until death occurred.

TEST 2 - SUBACUTE (LC_{50})

Procedure

Testing

The subacute dietary LC_{50} trial consisted of a 7-day quarantine and acclimation period, a 21-day dosing period, and a 7-day recovery period.

Sixty juvenile pastel mink were separated into 6 groups of 10 mink each. Each group consisted of 5 males and 5 females randomly chosen from healthy stock, and was approximately 8 months of age. One group was assigned to each of the following logarithmically scaled dietary concentrations (ppm) of DIMP: 0 (control), 1, 10, 100, 1000, and 10,000. Diet constituents and preparation procedures are given in Appendix I.

All animals in the subacute trial were housed indoors in an environmentally controlled cage room, at the Poultry Science Research and Teaching Center, Michigan State University. Each mink was housed individually in a 51 x 36 x 30 cm (length x width x height) cage equipped with water cup and feed container.

Feed was provided in removable containers attached to the inside of the cage on a swinging door such that feed consumption could be ascertained from measurement of unconsumed feed. Water was provided ad libitum.

During the 7-day predosing acclimation period all mink were provided with a control diet.

Body weights were recorded at the beginning of the dosing period and on days 7, 14, and 21 of dosing, and on day 7 of the recovery period (termination of the test).

Feed consumption was estimated by daily recovery of the unconsumed portion of a preweighed allotment of feed, and collectively weighed for each treatment level on days 7, 14, and 21 of dosing, and on day 7 of recovery.

Mortality, signs of intoxication, and behavioral changes were noted throughout both the dosing and recovery periods.

Blood for packed cell volume (hematocrit) and differential leukocyte counts was procured by toe-clip at the termination of the test. Blood was collected in heparinized microcapillary tubes (100 µl) and centrifuged for 7 minutes at 4500 rpm on an International Microcapillary Centrifuge[1] for hematocrit determination. Blood smears were allowed to air dry and were then fixed and stained in Wright's stain (see Appendix F). After staining, slides were first rinsed with phosphate buffer, for differentiation, and then with distilled water. They were then blotted and air dried. Differential leukocyte counts were made under oil immersion (930-x) and any abnormalities in cells were recorded.

At the end of the experiment animals were terminated by cervical dislocation, and necropsied. Gross pathomorphological observations were made, and the following organs were excised, weighed, and prepared for histopathological observation according to routine laboratory procedures: brain, heart, lungs, kidneys, spleen, and liver.

Statistical Analysis

Differences in body weight changes, feed consumption, hematocrit values, differential leukocyte counts, and organ weights were analyzed by a one-way analysis of variance and Dunnett's t-test. Zero predicted feed consumption was estimated by regression analysis.

Results

The determination of a subacute mean lethal dietary concentration of DIMP to mink was not possible since there was no significant mortality related to DIMP concentration in the diet (see Table 40). Only two animals died during the experiment. One was a female fed the control diet and the other was a female on the 1000 ppm diet. Both deaths resulted from wounds inflicted by neighboring mink which were able to squeeze under the partition between cages.

The mean of body weights recorded weekly throughout the experiment are shown in Table 41 and Figure 19. There were significantly lower mean body weights for the 10,000 ppm DIMP treatment group than for the control group on days 7, 14, and 21 of dosing. Although the 7-day post-treatment period showed a weight gain for these animals (DIMP 10,000 ppm) the mean body weight was still significantly depressed compared to the control.

Since mink have a high degree of variability in body weights, especially between sexes, the data in Table 41 may tend to obscure

---

[1] International Equipment Company, Boston, MA.

Table 40. Mortality associated with a subacute 21-day dietary administration of DIMP and a 7-day post-treatment recovery period.

Sex	Treatment (ppm)	No. of mink surviving during treatment				No. of mink surviving post-treatment	Mortality (%)
		1/15	1/22	1/29	2/5	2/13	
Male	DIMP 0	5	5	5	5	5	0
	1	5	5	5	5	5	0
	10	5	5	5	5	5	0
	100	5	5	5	5	5	0
	1000	5	5	5	5	5	0
	10000	5	5	5	5	5	0
Female	DIMP 0	5	5	4	4	4	20
	1	5	5	5	5	5	0
	10	5	5	5	5	5	0
	100	5	5	5	5	5	0
	1000	5	4	4	4	4	20
	10000	5	5	5	5	5	0
Combined Sexes	DIMP 0	10	10	9	9	9	10
	1	10	10	10	10	10	0
	10	10	10	10	10	10	0
	100	10	10	10	10	10	0
	1000	10	9	9	9	9	10
	10000	10	10	10	10	10	0

changes in body weight that might prove significant for one sex. Table 42 lists the mean percent change in body weight by sex over four weekly intervals. Highly significant losses ($P < 0.01$) in percent of body weight were recorded for both males and females fed 10,000 ppm DIMP during the first 7 days of dosing. A highly significant ($P < 0.01$) percent loss of body weight continued during the second week of dosing for these males. Females fed 10,000 ppm DIMP continued to lose weight ($P < 0.01$) only during the third week of dosing. It was also noted that the females fed the 10 ppm DIMP diet gained weight significantly over the controls during the second week of the test.

During the 7-day post-treatment period males on the 10,000 ppm DIMP diet gained weight significantly ($P < 0.01$) over the controls.

Feed consumption during the DIMP subacute trial is reported in Table 43. The mean feed consumption for the 21 days on treatment was significantly less for the mink on the 10,000 ppm DIMP treatment than for the control. Feed consumption was greater for this group than for the controls during the 7-day post-treatment period. Figure 20 predicts the extrapolated dose (in ppm) required for zero feed consumption. Based on regression analysis of data in Table 43 zero feed consumption would have occurred at a concentration greater than 100 percent DIMP, according to this analysis.

Table 44 shows the calculated average amount of DIMP ingested/kg body weight by the animals over the 21-day treatment period, based on mean feed consumption and mean body weight for the period. The animals on the 10,000 ppm DIMP treatment were calculated to have received a daily dose of DIMP more than 3 times the acute, oral LD_{50} as determined in Test 1.

The hematological parameters measured at the termination of the test are given in Table 45. Hematocrit (packed cell volume) was found to be significantly depressed ($P < 0.05$) for the animals on the 10,000 DIMP treatment. Differential leukocyte counts revealed a significantly lower percentage of lymphocytes in the peripheral blood of mink on the 10, 1000, and 10,000 ppm treatments. No consistent signs of intoxication were recorded for any treatment group on the DIMP subacute trial. However, the mink fed 10,000 DIMP behaved much more aggressively than animals on other treatments.

There were no consistent macroscopic lesions associated with a particular DIMP treatment at necropsy. No significant differences in organ weights of females were noted in any treatment group for brain, heart, lungs, or liver weights (see Table 46). However, there was a significant reduction in kidney weight for females on the 1 ppm DIMP diet. Male mink on the 1000 ppm DIMP treatment showed a significant decrease in lung weight (see Table 47). The male mink fed 10,000 ppm DIMP showed a significant decrease in heart, lung, kidney, and liver weights.

Table 41. Change in body weight of mink on 21-day dietary LC_{50} test and post-treatment recovery.

Treatment (ppm)	Mean body wt. (g)				
	Initial wt.	7 days	14 days	21 days	7 days post-treatment
DIMP 0	1346 ± 130[a]	1553 ± 156[a]	1572 ± 168[a]	1560 ± 165[a]	1663 ± 191[a]
1	1261 ± 133[a]	1466 ± 152[a]	1422 ± 153[a]	1496 ± 164[a]	1479 ± 151[a]
10	1301 ± 148[a]	1432 ± 157[a]	1436 ± 150[a]	1480 ± 153[a]	1466 ± 128[a]
100	1493 ± 149[a]	1655 ± 159[a]	1583 ± 150[a]	1642 ± 158[a]	1603 ± 145[a]
1000	1155 ± 96.4[a]	1334 ± 114[a]	1344 ± 124[a]	1401 ± 141[a]	1362 ± 129[a]
10000	1212 ± 52[a]	1154 ± 54[b]	1060 ± 44[b]	1047 ± 49[b]	1125 ± 51[b]

[a] Means with the same superscript are not significantly different from the controls.

[b] Means significantly different from control at $P < 0.05$ level of significance.

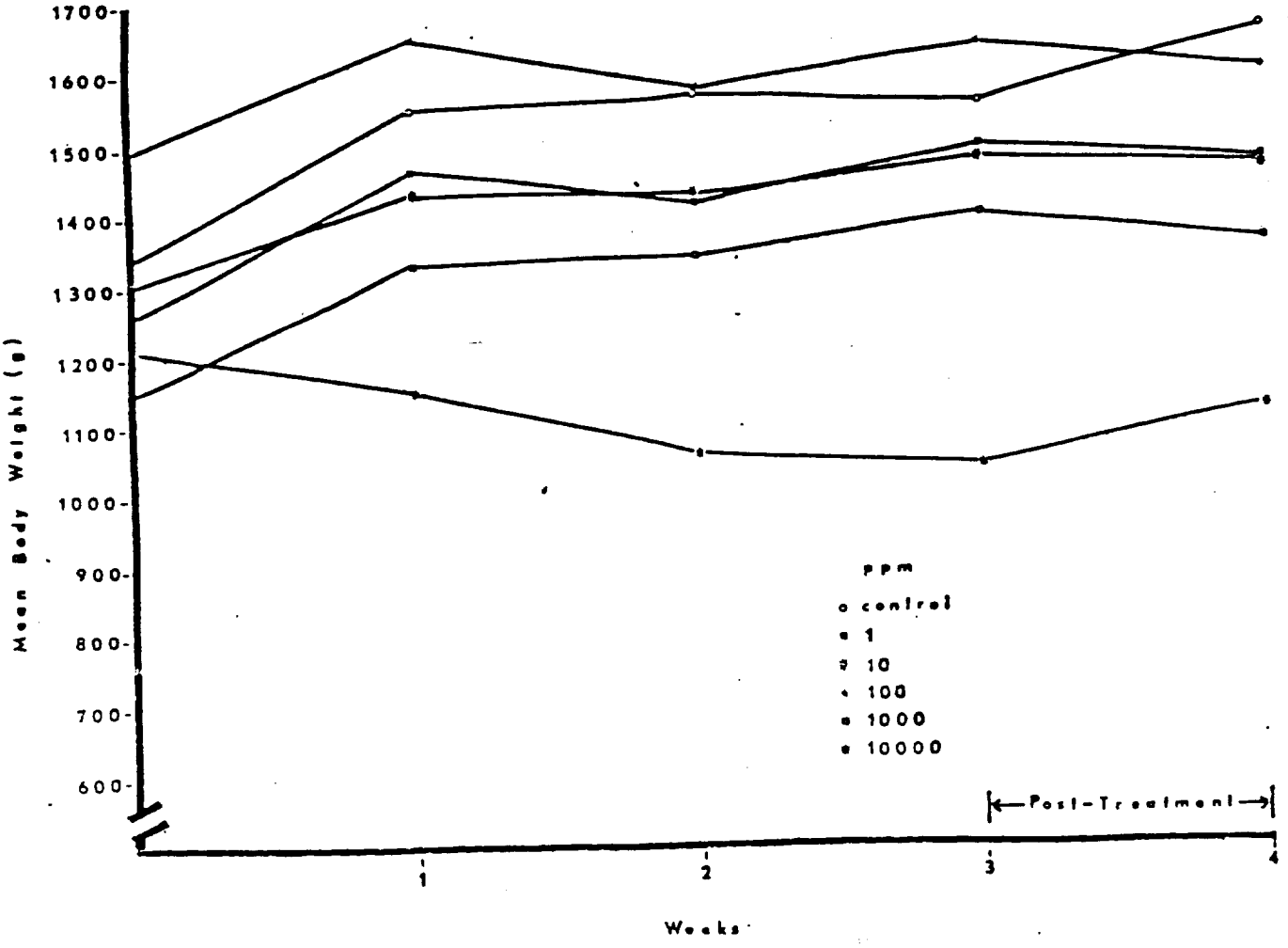

Figure 19. Mean body weights of mink on the 21-day subacute test fed DIMP at various levels.

Table 42. Effect of subacute dietary DIHP administration upon percent change in mink body weight taken at weekly intervals.

Sex	Treatment (ppm)	Treatment N	Treatment 1/15-1/22 % Gain (loss) in body wt.	Treatment N	Treatment 1/23-1/29 % Gain (loss) in body wt.	Treatment N	Treatment 1/30-2/5 % Gain (loss) in body wt.	Post-treatment N	Post-treatment 2/6-2/13 % Gain (loss) in body wt.
Male	DIHP 0	5	$17.2 \pm 2.42_a$ (1)	5	$(1.1 \pm 1.71)_a$ (1)	5	$(2.7 \pm 3.34)_a$ (1)	5	$(2.8 \pm 1.59)_a$ (1)
	1	5	$17.6 \pm 4.48_a$	5	$(1.0 \pm 2.40)_a$	5	$6.2 \pm 1.49_a$	5	$(3.6 \pm 1.95)_a$
	10	5	$12.9 \pm 3.66_a$	5	$(1.3 \pm 0.92)_a$	5	5.4 ± 2.87	5	$(4.3 \pm 1.01)_a$
	100	5	$10.1 \pm 5.72_a$	5	$(4.2 \pm 4.21)_a$	5	$3.5 \pm 4.78_a$	5	$(3.4 \pm 1.20)_a$
	1000	5	$17.7 \pm 4.37_a$	5	$4.1 \pm 3.24_a$	5	$8.0 \pm 2.61_a$	5	$(4.0 \pm 1.61)_a$
	10000	5	$(1.9 \pm 1.47)_c$	5	$(10.3 \pm 1.07)_c$	5	$0.7 \pm 1.23_a$	5	$6.8 \pm 1.53_c$
Female	DIHP 0	5	$13.0 \pm 4.20_a$ (1)	4	$(3.6 \pm 1.02)_a$ (1)	4	$3.2 \pm 1.38_a$ (1)	4	$3.1 \pm 0.66_a$ (1)
	1	5	$15.4 \pm 1.51_a$	5	$(4.8 \pm 1.44)_a$	5	$4.1 \pm 2.70_a$	5	$3.6 \pm 2.75_a$
	10	5	$8.4 \pm 4.18_a$	5	$3.3 \pm 1.66_b$	5	$0.7 \pm 1.65_a$	5	$5.7 \pm 1.62_a$
	100	5	$14.1 \pm 4.03_a$	5	$(3.8 \pm 1.40)_a$	5	$4.5 \pm 1.06_a$	5	$(0.3 \pm 1.65)_a$
	1000	5	$6.8 \pm 2.72_a$	4	$(4.6 \pm 2.78)_a$	4	$(2.0 \pm 3.91)_a$	4	$0.1 \pm 1.66_a$
	10000	5	$(7.7 \pm 1.98)_c$	5	$(5.5 \pm 1.14)_a$	5	$(3.6 \pm 0.89)_c$	5	$8.5 \pm 2.20_a$
Combined Sexes	DIHP 0	10	$15.1 \pm 2.52_a$ (1)	9	$(2.6 \pm 1.13)_a$ (1)	9	$(0.1 \pm 2.18)_a$ (1)	8	$0.1 \pm 1.35_a$ (1)
	1	10	$16.5 \pm 2.38_a$	10	$(3.3 \pm 1.47)_a$	10	$5.1 \pm 1.58_a$	10	$0.0 \pm 2.03_a$
	10	10	$10.6 \pm 2.87_a$	10	$1.0 \pm 1.20_a$	10	$3.1 \pm 1.76_a$	10	$0.7 \pm 1.85_a$
	100	10	$12.1 \pm 3.56_a$	10	$(4.0 \pm 2.22)_a$	10	$4.0 \pm 2.45_a$	10	$(1.8 \pm 1.13)_a$
	1000	10	$12.3 \pm 3.10_a$	9	$0.3 \pm 2.22_a$	9	$3.6 \pm 2.80_a$	9	$(2.2 \pm 1.34)_a$
	10000	10	$(4.8 \pm 1.54)_c$	10	$(7.9 \pm 1.09)_a$	10	$(1.4 \pm 1.02)_a$	10	$7.7 \pm 1.37_c$

(1) Means with same subscript are not significantly different from control ($P > 0.05$).

b Means significantly different from control ($P < 0.05$).

c Means significantly different from control ($P < 0.01$).

Table 43. Feed consumption of mink on 21-day dietary LC_{50} test and post-treatment recovery period.

Treatment (ppm)	Feed consumption (g/mink/day) 1/15-1/22	1/23-1/29	1/30-2/5	Mean for 21 days on treatment ± S.E.	Post-treatment 2/6-2/13
DIMP 0	323	290	262	291.7 ± 17.6	261
1	307	287	281	291.7 ± 7.9	224
10	268	276	273	272.3 ± 2.3	246
100	312	259	266	279.0 ± 16.6	248
1000	262	278	264	268.5 ± 5.0	251
10000	157	177	270	201.3 ± 34.8[a]	290

[a] Significantly different from control ($P < 0.05$)

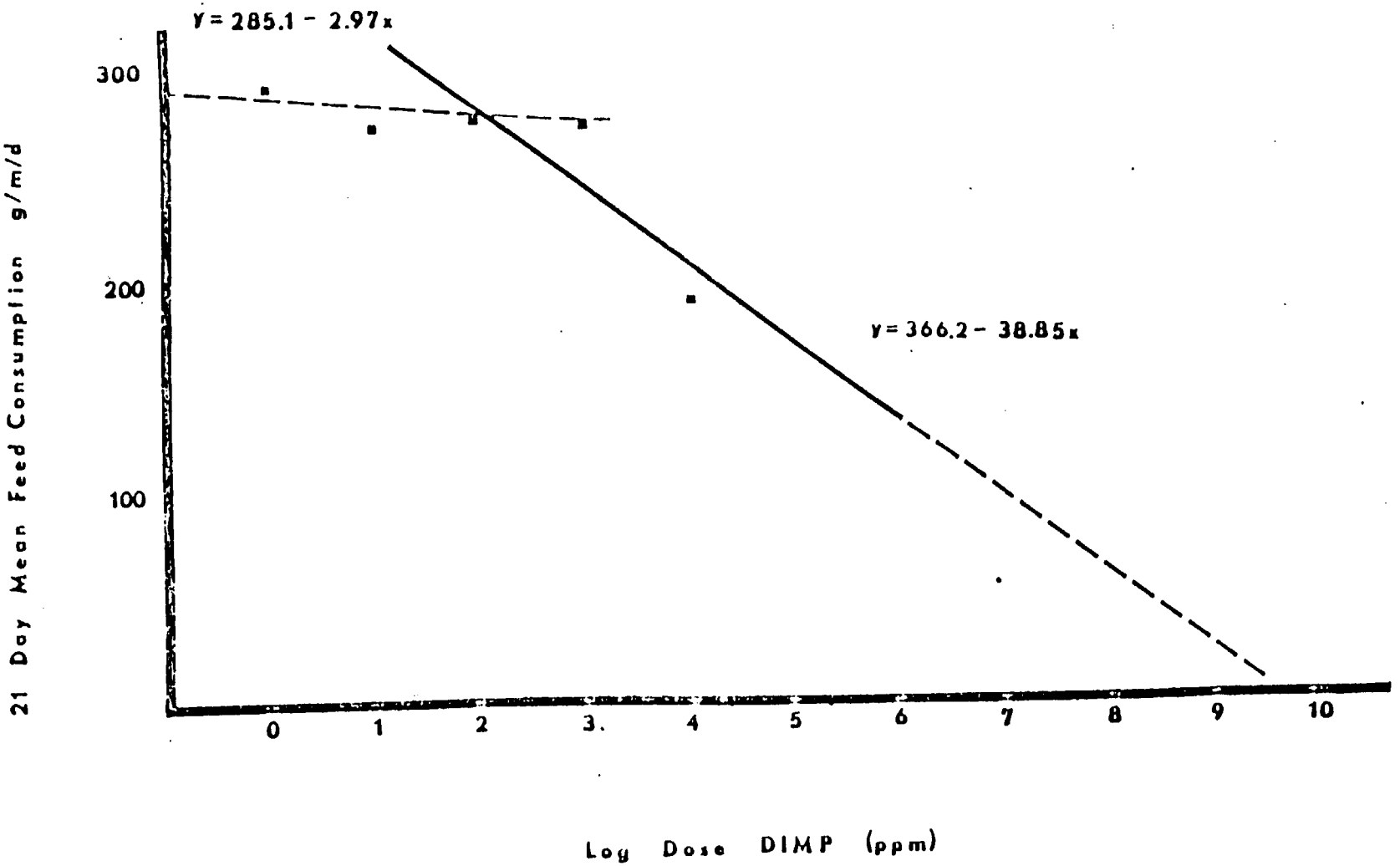

Figure 20. Regression lines for the data presented in Table 43 in the regression equations x = log dose DIMP in ppm, y = mean feed consumption for 21 days, in g/mink/day.

Table 44. Feed consumption, body weight, and amount of chemical ingested by adult mink fed DIMP at various levels for 21 days.

DIMP in diet (ppm)	Feed consumed (g/mink/day)	DIMP consumed (mg/mink/day)	Mean body wt. (g)	DIMP consumed (mg/kg/day)
0	291.7	0	1561.7	0
1	291.7	0.292	1461.3	0.200
10	272.3	2.723	1449.3	1.879
100	279.0	27.9	1626.6	17.159
1000	273.5	273.5	1359.6	201.16
10000	201.3	201.3	1087.0	1851.9

TABLE 45. Effect of subacute dietary DIMP upon mink hematocrit values and differential leukocyte counts.

Treatment (ppm)	N	Hematocrit[a]	Leukocyte cell type (% ± S.E.)					
			Basophils	Eosinophils	Band-neutrophils	Segmented neutrophils	Lymphocytes	Monocytes
DIMP 0	9	56.5 ± 0.72[b]	0.3 ± 0.16	3.1 ± 1.06	0.0 ± 0.00	57.4 ± 3.32	34.7 ± 2.76	4.4 ± 0.90
1	10	56.7 ± 1.00	0.4 ± 0.22	2.2 ± 0.60	0.0 ± 0.00	66.7 ± 3.78	26.2 ± 3.04	4.4 ± 0.64
10	10	57.7 ± 1.14	0.3 ± 0.20	2.9 ± 0.92	0.1 ± 0.09	71.1 ± 3.20	22.8 ± 2.86**	2.7 ± 0.47
100	10	56.4 ± 0.92	0.6 ± 0.20	2.6 ± 0.40	0.8 ± 0.44	63.1 ± 2.61	30.2 ± 2.13	2.8 ± 0.61
1000	9	51.4 ± 2.03	0.1 ± 0.08	1.8 ± 0.49	0.4 ± 0.28	68.9 ± 2.52	25.9 ± 1.97*	2.7 ± 0.22
10000	10	52.7 ± 1.03	0.2 ± 0.13	0.8 ± 0.19	0.1 ± 0.09	69.9 ± 3.04	22.1 ± 1.74**	3.8 ± 0.88

a. N= 8 for 1000 ppm DIMP treatment

b. mean ± S.E.

* Treatment mean significantly different from control mean ($P < 0.05$).

** Treatment mean significantly different from control mean ($P < 0.01$).

Table 46 . Effect of subacute dietary DIMP upon female mink organ weights.

Treatment (ppm)	N	Organ weight (g ± S.E.)				
		Brain	Heart	Lungs	Kidneys	Liver[a]
DIMP 0	5	8.3 ± 0.12	7.0 ± 0.31	6.5 ± 1.39	4.2 ± 0.21	36.9 ± 2.73
1	5	7.8 ± 0.33	7.4 ± 0.41	7.4 ± 0.63	3.2 ± 0.13*	32.5 ± 1.52
10	5	9.0 ± 0.38	6.5 ± 0.31	6.5 ± 0.29	3.4 ± 0.36	38.1 ± 1.92
100	5	8.7 ± 0.32	6.8 ± 0.45	6.8 ± 0.24	3.3 ± 0.30	33.5 ± 3.81
1000	5	8.2 ± 0.45	6.1 ± 0.31	6.5 ± 0.13	3.7 ± 0.35	33.3 ± 2.14
10000	5[b]	8.4 ± 0.08	7.1 ± 0.32	6.4 ± 0.18	5.2 ± 0.59	31.4 ± 6.48

[a] N=4 for 1000 ppm DIMP treatment

[b] Two females died prior to termination of the test.

* Treatment mean significantly different from control ($P < 0.05$).

Table 47. Effect of subacute dietary DIMP upon male organ weights.

Treatment (ppm)	N	Mean weight (g ± S.E.)					
		Brain	Heart	Lungs	Kidneys	Spleen	Liver
DIMP 0	5	10.8 ± 0.21	14.2 ± 1.20	11.2 ± 0.48	9.5 ± 0.61	4.5 ± 0.51	55.9 ± 5.23
1	5	10.5 ± 0.27	13.6 ± 1.05	11.2 ± 0.95	8.5 ± 0.52	4.5 ± 0.40	51.2 ± 4.16
10	5	11.0 ± 0.62	13.7 ± 0.76	10.2 ± 0.48	8.7 ± 0.43	4.1 ± 0.33	46.5 ± 3.09
100	5	10.4 ± 0.35	11.9 ± 0.71	10.3 ± 0.42	9.3 ± 0.10	4.3 ± 0.18	49.3 ± 1.23
1000	5	10.4 ± 0.16	11.3 ± 0.57	9.0 ± 0.36*	8.5 ± 0.23	5.7 ± 0.71	45.2 ± 1.72
10000	5[a]	10.0 ± 0.31	10.7 ± 0.68*	8.5 ± 0.33**	6.7 ± 0.22**	4.5 ± 0.58	37.6 ± 2.33*

* Treatment mean significantly different from control ($P < 0.05$).

** Treatment mean significantly different from control ($P < 0.01$).

a. Four males died, prior to termination of the test.

Discussion

Since no determination of lethal concentration of dietary DIMP to mink could be made at the concentrations and length of exposure used, DIMP was considered to be nontoxic to mink by ingestion in the 21-day test. Although weight loss was noted for the animals receiving the highest dietary concentration (10,000 ppm), the reduced feed consumption by these animals while on the test diet may have been responsible for the weight loss. The increase in feed consumption and body weight displayed by these animals during the post-treatment period (when they were placed on the control diet), suggests a palatability problem with DIMP in high dietary concentrations. The marked aggressiveness of the animals probably due to hunger during the dosing period and the regression to zero feed consumption at a concentration greater than 100 percent DIMP also support this contention.

The calculated average daily ingestion of DIMP by animals on the 100 and 10,000 ppm diets suggests that DIMP is not a cumulative poison, since the total dose over the 21-day period would have far exceeded the acute, oral LD_{50} calculated in the acute toxicity experiment. The rapid metabolism and excretion of DIMP by orally dosed Mallard ducks and Bobwhite quail discussed elsewhere in this report, and the fact that three times the acute lethal dose was consumed by the mink on the highest concentration (DIMP 10,000 ppm) suggest that it is similarly metabolized by these species.

Hematological parameters of mink fed DIMP on a subacute basis were altered at the high dietary concentrations. The decreased hematocrit noted for the 10,000 ppm DIMP treatment animals may have been caused by either decreased erythropoiesis or by increased clearance of erythrocytes. The decrease in hematocrit values found in animals on the 10,000 ppm DIMP treatment may have been due to decreased erythropoiesis from protein deficiency (decreased feed consumption). Since a "pair fed" control was not a part of the protocol, this is merely speculation. Control hematocrit values closely matched the values reported by other workers (Asher et al., 1976; Skrede, 1970) for mink. Percentage lymphocyte depression in the 10, 1000, and 10,000 ppm treatments may have been dose related even though the animals on the 100 ppm DIMP diet failed to show this difference. Since a total white cell count was not made on the blood taken from these animals, it is difficult to determine whether this shift in lymphocyte numbers was absolute or relative. The values for the leukocyte cell types for these animals and for the controls were in reasonable agreement with values established by Fletch and Karstad, (1972); Asher et al., (1976); and Glibert, (1969).

Organ weight differences noted at necropsy for male animals on the 10,000 ppm DIMP treatment were dose related. The difference in kidney weight in female animals on the 1 ppm DIMP diet was probably an artifact associated with chance variation on sampling error, since animals fed the 10, 100, 1000, or 10,000 ppm DIMP diets failed to show a similar difference. The significant depression in male organ weights associated with the 10,000 ppm DIMP treatment may have

been due to decreased feed consumption and body weight gain (Shärer, 1977), and not necessarily a function of toxicosis. Since a pair-fed control was not maintained, the cause of these organ weight differences is difficult to ascertain. Control mink organ weights were not appreciably different than mink organ weights reported by Wood et al. (1965).

Since no consistent gross pathological changes were noted for any treatment group, it cannot be conclusively stated that the body weight depression noted for 10,000 ppm DIMP treatment animals was toxicant related.

TEST 3 - CHRONIC

Procedure

Testing

The chronic toxicity feeding study began with 120 immature dark variety mink (approximately 3 months of age) and continued through one reproductive season (12 months total duration). Four groups of 30 randomly selected animals (six males and 24 females per group) were used in the test. The following concentrations (ppm) of DIMP in the diet were fed (1 group per concentration level): 0 control; 50; 150; and 450. The diet constituents and feed prepartation procedures are given in Appendix I. Water was provided ad libitum.

Animals were housed out-of-doors in commercial style mink ranch sheds at the experimental facilities of the Fur Animal Project, Department of Poultry Science, Michigan State University. The animals fed each diet were assigned individually to single-tier cages 61 x 46 x 30 cm (length x width x height) or to double tiered cages 61 x 30 x 30 cm (length x width x height) plus a top nest box tier 38 x 30 x 30 cm (length x width x height) in 6 subgroups of 5 animals (one male, four females) per subgroup. The subgroups were randomly placed in one of three sheds. Each of the subgroups was specified by color coded mink identification cards placed above the cages which matched the color coding on the respective feed containers.

During the reproductive season, the females were housed individually in breeder cages 76 x 61 x 46 cm (length x width x height) to which a nest box 30 x 25 x 25 cm (length x width x height) was attached to the outside of the cage.

Bedding, consisting of shredded wood, was provided for insulation in the winter and for nesting during the reproductive season.

Mortality and signs of intoxication were recorded throughout the experiment.

Body weight measurements were made at two week intervals, except during the gestation period.

Feed consumption was estimated once every two weeks for 5 months by weighing unconsumed feed recovered from a preweighed allotment given the previous day, for each animal.

Blood for hematocrit (packed cell volume), hemoglobin, and blood smears was collected by toe-clip at the beginning of the experiment at 3 month intervals (except during the gestation period), and at the termination of the test.

Hematocrit values were determined from blood drawn into heparinized microcapillary tubes (100 µl) and centrifuged in an International Microcapillary Centrifuge[1] for 7 minutes at 4500 rpm.

Hemoglobin values were determined by the cyanmethemoglobin method, based on a quantitative spectrographic change in absorption of light relating to hemoglobin concentration (see Appendix E: Determination of Hemoglobin Concentration).

Blood smears were allowed to air dry and were then fixed and stained with Wright's stain (see Appendix F). After staining, the slides were rinsed with phosphate buffer for differentiation, followed by distilled water. They were then blotted and air dried. Differential leukocyte counts were made on the smears collected at the termination of the test. Counting was done under oil immersion and abnormalities in cell types were recorded.

Mink mating was initiated on March 1, 1978, and lasted approximately 20 days. Females were bred to males within their respective treatment group whenever possible. Breeding attempts began at 7:00 a.m. daily and were ceased at noon. Females were introduced into the males' cages every fourth day for one half of an hour to an hour, until a positive mating was secured. Positive matings were confirmed by checking post-coital vaginal aspirations for sperm. Positive matings were followed-up by a second mating attempt eight days later.

After breeding, the females were transferred to the cages described above for whelping.

During the whelping period (April 20 - May 15), the nest boxes were checked daily for evidence of whelping. Newborn kits were sexed and weighed on the day of whelping and at one month of age. Whelping females were also weighed on the day of whelping and one month after whelping.

Length of gestation, litter size, sex ratio, kit mortality, increase in kit "biomass" during lactation, and lactating female weight changes were recorded.

At the termination of the chronic test, the mink were weighed, and blood samples were taken (by cardiac puncture) and stored for future analysis.

---

[1] International Equipment Company, Boston, MA.

The animals were terminated by cervical dislocation, and were then necropsied. Any gross pathomorphological changes were recorded. The following organs were then excised and weighed: brain, liver, kidneys, spleen, gonads, lungs, heart, and adrenal glands. Portions of these organs, in addition to portions of the intestine, stomach, skeletal muscle, adipose tissue, and integument were then fixed in 10% neutral, buffered formalin and prepared for histopathological examination according to routine histological procedures.

Statistical Analysis

All parameters were analyzed for significant differences by analysis of variance and Dunnett's t-test.

Results

Chronic ingestion of dietary DIMP by mink for 12 months resulted in greater mortality for the females on the DIMP-treated diets than for those fed the control diet (Table 48). Insufficient numbers of males were utilized to reveal a difference in male mortality.

Body weight measurements revealed no significant differences for any treatment with respect to controls, for any of the measurement periods (Table 49). Percent change in body weight also failed to show a consistent difference in treatments as compared to controls (Table 50).

Feed consumption by animals on the DIMP diets was not significantly different from controls for most measurement periods (Table 51). Significant differences in feed consumption appeared in only two instances. In one case the depressed feed consumption did not appear to be dose related (50 ppm DIMP treatment on September 1, 1977); in the other case, depressed feed consumption may have been dose related (450 ppm DIMP treatment on November 15, 1977) but was not trend oriented when compared to feed consumption of other treatments on the same date.

An estimated daily ingested dose of DIMP (as calculated from body weight and feed consumption) by mink on each treatment is shown in Table 52.

Analysis of the data collected on hematological parameters at the termination of the test revealed increased hematocrit values for males on the 150 ppm and 450 ppm DIMP treatments (see Table 53). Significant differences in hemoglobin content or mean corpuscular hemoglobin concentration were not found in any treatment groups with respect to control values (Tables 54 and 55).

Differential leukocyte counts revealed no differences among DIMP treatments consistent with toxicosis (Table 56).

Reproductive success of mink on the various DIMP treatments is shown in Table 57. No adverse effects upon whelping rates,

Table 48. Mortality of mink fed DIMP at various levels for 12 months.

Sex	Treatment (ppm)	Mortality by date			
		7/21/77	10/18/77	1/17/78	6/30/78
Male	DIMP 0	0/6	1/6	1/6	1/6
	50	0/6	1/6	1/6	1/6
	150	0/6	0/6	0/6	0/6
	450	0/6	0/6	0/6	0/6
Female	DIMP 0	0/24	0/24	0/24	0/24
	50	0/24	1/23*	1/23*	2/23*
	150	0/24	0/24	0/24	3/24
	450	0/24	0/24	2/24	5/24
Combined Sexes	DIMP 0	0/30	1/30	1/30	1/30
	50	0/30	2/29*	2/29*	3/29
	150	0/30	0/30	0/30	3/30
	450	0/30	0/30	2/30	5/30

* One mink on this treatment escaped.

Table 49. Effect of chronic dietary administration of DIMP to male and female mink upon body weight (g ± S.E.) gain by date.

Sex	Treatment (ppm)	N	7/21/77	N	8/3/77	N	8/18/77	N	9/1/77	N	9/15/77	N	9/29/77
Males	DIMP 0	6	1137 ± 65$_a$ [1]	6	1339 ± 73$_a$	6	1378 ± 98$_a$	5	1569 ± 112$_a$	5	1591 ± 131$_a$	5	1669 ± 110$_a$
	50	6	1072 ± 34$_a$	6	1213 ± 55$_a$	6	1338 ± 63$_a$	6	1482 ± 50$_a$	5	1554 ± 50$_a$	5	1608 ± 51$_a$
	150	6	1101 ± 20$_a$	6	1293 ± 27$_a$	6	1395 ± 28$_a$	6	1513 ± 29$_a$	6	1515 ± 44$_a$	6	1615 ± 51$_a$
	450	6	1048 ± 40$_a$	6	1224 ± 51$_a$	6	1344 ± 55$_a$	6	1520 ± 68$_a$	6	1565 ± 74$_a$	6	1720 ± 81$_a$
Females	DIMP 0	24	750 ± 15$_a$	24	876 ± 18$_a$	24	934 ± 20$_a$	24	955 ± 23$_a$	24	984 ± 23$_a$	24	1039 ± 28$_a$
	50	24	761 ± 17$_a$	24	870 ± 25$_a$	24	957 ± 24$_a$	24	952 ± 24$_a$	24	965 ± 29$_a$	24	1016 ± 30$_a$
	150	24	771 ± 13$_a$	24	884 ± 17$_a$	24	935 ± 20$_a$	24	926 ± 22$_a$	24	957 ± 23$_a$	24	996 ± 19$_a$
	450	24	753 ± 16$_a$	24	856 ± 20$_a$	24	911 ± 24$_a$	24	911 ± 26$_a$	24	945 ± 26$_a$	24	1001 ± 28$_a$
Combined Sexes	DIMP 0	30	828 ± 33$_a$	30	969 ± 39$_a$	30	1023 ± 40$_a$	29	1061 ± 51$_a$	29	1089 ± 52$_a$	29	1147 ± 53$_a$
	50	30	823 ± 27$_a$	30	945 ± 33$_a$	30	1033 ± 38$_a$	30	1058 ± 44$_a$	29	1066 ± 48$_a$	29	1118 ± 49$_a$
	150	30	837 ± 27$_a$	30	966 ± 33$_a$	30	1027 ± 32$_a$	30	1044 ± 47$_a$	30	1069 ± 45$_a$	30	1120 ± 49$_a$
	450	30	812 ± 26$_a$	30	930 ± 33$_a$	30	998 ± 39$_a$	30	1032 ± 51$_a$	30	1069 ± 52$_a$	30	1145 ± 59$_a$

[1] Means in the same column with the same subscript are not significantly different from their respective control values ($P > 0.05$).

Continued

Table 49. Continued

Sex	Treatment (ppm)	N	10/13/77	N	10/27/77	N	11/10/77	N	11/22/77	N	12/8/77	N	12/23/77
Males	DINP 0	5	1686 ± 141$_a$	5	1710 ± 167$_a$	5	1781 ± 154$_a$	5	1732 ± 181$_a$	5	1564 ± 172$_a$	5	1596 ± 185$_a$
	50	5	1631 ± 66$_a$	5	1677 ± 80$_a$	5	1755 ± 70$_a$	5	1728 ± 84$_a$	5	1633 ± 73$_a$	5	1674 ± 59$_a$
	150	6	1583 ± 38$_a$	6	1582 ± 47$_a$	6	1663 ± 54$_a$	6	1630 ± 41$_a$	6	1468 ± 66$_a$	6	1452 ± 105$_a$
	450	6	1695 ± 81$_a$	6	1704 ± 77$_a$	6	1778 ± 73$_a$	6	1815 ± 65$_a$	6	1669 ± 51$_a$	6	1728 ± 56$_a$
Females	DINP 0	24	1066 ± 27$_a$	24	1065 ± 28$_a$	23	1058 ± 28$_a$	24	1041 ± 31$_a$	24	968 ± 28$_a$	24	1018 ± 28$_a$
	50	24	1039 ± 30$_a$	22	1008 ± 26$_a$	22	1035 ± 26$_a$	22	1017 ± 24$_a$	22	972 ± 23$_a$	22	1006 ± 24$_a$
	150	24	1011 ± 22$_a$	24	979 ± 24$_a$	24	1004 ± 22$_a$	24	1004 ± 23$_a$	24	930 ± 24$_a$	24	980 ± 28$_a$
	450	24	1018 ± 25$_a$	24	995 ± 24$_a$	24	1007 ± 27$_a$	23	1007 ± 31$_a$	23	940 ± 27$_a$	22	1009 ± 25$_a$
Combined Sexes	DINP 0	29	1173 ± 54$_a$	29	1177 ± 58$_a$	28	1187 ± 64$_a$	29	1160 ± 59$_a$	29	1071 ± 56$_a$	29	1118 ± 57$_a$
	50	29	1141 ± 50$_a$	27	1132 ± 56$_a$	27	1168 ± 59$_a$	27	1149 ± 59$_a$	27	1094 ± 55$_a$	27	1130 ± 55$_a$
	150	30	1125 ± 46$_a$	30	1099 ± 49$_a$	30	1136 ± 52$_a$	30	1129 ± 50$_a$	30	1037 ± 46$_a$	30	1074 ± 46$_a$
	450	30	1153 ± 56$_a$	30	1137 ± 57$_a$	30	1161 ± 62$_a$	29	1174 ± 67$_a$	29	1091 ± 44$_a$	28	1199 ± 44$_a$

Continued

Table 49. Continued

Sex	Treatment (ppm)	N	1/8/78	N	1/18/78	N	2/4/78	N	2/19/78	N	3/4/78	N	6/30/78
Males	DIMP 0	5	1483 ± 190$_a$	5	1476 ± 163$_a$	5	1496 ± 194$_a$	5	1607 ± 190$_a$	5	1595 ± 167$_a$	5	1692 ± 112$_a$
	50	5	1556 ± 63$_a$	5	1578 ± 72$_a$	5	1588 ± 111$_a$	5	1618 ± 131$_a$	5	1638 ± 151$_a$	5	1552 ± 76$_a$
	150	6	1437 ± 93$_a$	6	1417 ± 104$_a$	6	1458 ± 125$_a$	6	1533 ± 115$_a$	6	1523 ± 100$_a$	6	1578 ± 70$_a$
	450	6	1628 ± 53$_a$	6	1638 ± 43$_a$	6	1722 ± 55$_a$	6	1733 ± 60$_a$	6	1750 ± 65$_a$	6	1623 ± 99$_a$
Females	DIMP 0	24	970 ± 31$_a$	24	962 ± 26$_a$	24	941 ± 24$_a$	24	968 ± 64$_a$	24	965 ± 22$_a$	24	821 ± 29$_a$
	50	22	916 ± 26$_a$	22	927 ± 22$_a$	21	922 ± 21$_a$	22	934 ± 21$_a$	22	929 ± 23$_a$	21	844 ± 31$_a$
	150	24	911 ± 27$_a$	24	899 ± 29$_a$	24	871 ± 31$_a$	24	911 ± 26$_a$	24	922 ± 23$_a$	19	840 ± 25$_a$
	450	22	963 ± 26$_a$	22	948 ± 26$_a$	22	944 ± 28$_a$	22	971 ± 29$_a$	22	969 ± 27$_a$	19	832 ± 26$_a$
Combined Sexes	DIMP 0	29	1058 ± 55$_a$	29	1051 ± 50$_a$	29	1036 ± 55$_a$	29	1078 ± 59$_a$	29	1074 ± 56$_a$	29	971 ± 68$_a$
	50	27	1035 ± 54$_a$	27	1047 ± 54$_a$	26	1050 ± 58$_a$	27	1061 ± 59$_a$	27	1060 ± 63$_a$	27	980 ± 62$_a$
	150	30	1016 ± 48$_a$	30	1003 ± 49$_a$	30	988 ± 56$_a$	30	1036 ± 55$_a$	30	1042 ± 52$_a$	25	1017 ± 70$_a$
	450	28	1106 ± 57$_a$	28	1096 ± 58$_a$	28	1111 ± 65$_a$	28	1143 ± 67$_a$	28	1136 ± 66$_a$	25	1022 ± 74$_a$

Table 50. Effect of chronic dietary administration of DIMP to male and female mink upon mean percent change in body weight (g ± S.E.) by date.

Sex	Treatment (ppm)	N	7/21/77-8/3/77	N	8/4/77-8/18/77	N	8/19/77-9/1/77	N	9/2/77-9/15/77
Males	DIMP 0	6	$18.0 \pm 1.21_a$ [1]	6	$4.3 \pm 3.60_a$	5	$9.7 \pm 1.61_a$	5	$1.0 \pm 1.64_a$
	50	6	$12.9 \pm 2.95_a$	6	$10.3 \pm 1.36_a$	6	$11.4 \pm 3.05_a$	5	$2.8 \pm 0.86_a$
	150	6	$17.4 \pm 0.97_a$	6	$8.0 \pm 0.85_a$	6	$8.5 \pm 1.43_a$	6	$0.1 \pm 1.22_a$
	450	6	$17.0 \pm 3.50_a$	6	$9.9 \pm 1.13_a$	6	$12.8 \pm 1.62_a$	6	$2.9 \pm 0.96_a$
Females	DIMP 0	24	$16.9 \pm 1.05_a$	24	$5.8 \pm 1.06_a$	24	$2.0 \pm 0.99_a$	24	$2.9 \pm 1.03_a$
	50	24	$15.0 \pm 1.06_a$	24	$9.4 \pm 1.18_a$	24	$(0.3 \pm 0.85)_a$	24	$1.2 \pm 1.03_a$
	150	24	$14.4 \pm 0.89_a$	24	$6.1 \pm 0.99_a$	24	$(0.9 \pm 0.99)_a$	24	$3.4 \pm 0.97_a$
	450	24	$13.6 \pm 1.03_a$	24	$6.4 \pm 0.88_a$	24	$(0.1 \pm 0.83)_a$	24	$3.9 \pm 0.73_a$
Combined Sexes	DIMP 0	30	$17.1 \pm 0.88_a$	30	$5.5 \pm 1.12_a$	29	$3.3 \pm 1.02_a$	29	$2.6 \pm 0.91_a$
	50	30	$14.6 \pm 1.04_a$	30	$9.6 \pm 0.99_b$	30	$2.0 \pm 1.25_a$	29	$1.5 \pm 0.87_a$
	150	30	$15.0 \pm 0.77_a$	30	$6.4 \pm 0.82_a$	30	$1.0 \pm 1.09_a$	30	$2.8 \pm 0.85_a$
	450	30	$14.3 \pm 1.11_a$	30	$7.1 \pm 0.78_a$	30	$2.5 \pm 1.19_a$	30	$3.7 \pm 0.62_a$

[1] Means in the same column with the same subscript are not significantly different from their respective control values ($P > 0.05$).

Continued

Table 50. Continued

Sex	Treatment	N	9/16/77-9/29/77	N	9/30/77-10/13/77	N	10/14/77-10/27/77	N	10/28/77-11/10/77
Male	DIMP 0	5	$6.4 \pm 1.69_a$	5	$0.4 \pm 2.01_a$	5	$1.0 \pm 1.90_a$	5	$4.6 \pm 1.34_a$
	50	5	$5.3 \pm 1.29_a$	5	$(0.4 \pm 1.76)_a$	5	$2.8 \pm 1.61_a$	5	$4.9 \pm 1.42_a$
	150	6	$6.6 \pm 0.79_a$	6	$(1.6 \pm 2.64)_a$	6	$0.2 \pm 0.99_a$	4	$5.6 \pm 1.94_a$
	450	6	$9.9 \pm 0.78_a$	6	$(1.4 \pm 1.15)_a$	6	$0.6 \pm 0.72_a$	6	$4.6 \pm 2.21_a$
Female	DIMP 0	24	$5.5 \pm 1.28_a$	24	$2.8 \pm 0.73_a$	24	$(0.1 \pm 0.88)_a$	23	$0.7 \pm 1.05_a$
	50	24	$5.6 \pm 1.10_a$	24	$2.3 \pm 0.94_a$	22	$(0.7 \pm 0.85)_a$	22	$2.9 \pm 0.98_a$
	150	24	$4.4 \pm 0.92_a$	24	$1.7 \pm 1.67_a$	24	$(3.4 \pm 0.95)_b$	24	$1.6 \pm 1.42_a$
	450	24	$6.0 \pm 0.71_a$	24	$2.0 \pm 1.00_a$	24	$(2.1 \pm 0.87)_a$	24	$1.1 \pm 0.83_a$
Combined Sexes	DIMP 0	29	$5.6 \pm 1.10_a$	29	$2.4 \pm 0.71_a$	29	$0.1 \pm 0.80_a$	28	$1.4 \pm 1.36_a$
	50	29	$5.5 \pm 0.93_a$	29	$1.9 \pm 0.86_a$	27	$(0.0 \pm 0.80)_a$	27	$3.3 \pm 0.85_a$
	150	30	$4.8 \pm 0.77_a$	30	$1.0 \pm 1.46_a$	30	$(2.8 \pm 0.82)_b$	28	$2.2 \pm 1.27_a$
	450	30	$6.8 \pm 0.66_a$	30	$1.3 \pm 0.87_a$	30	$(1.6 \pm 0.74)_a$	30	$1.8 \pm 0.84_a$

Continued

Table 50. Continued

Sex	Treatment	N	11/11/77-11/22/77	N	11/23/77-12/8/77	N	12/9/77-12/23/77
Male	DIMP 0	5	$(3.5 \pm 2.67)_a$	5	$(9.9 \pm 1.17)_a$	5	$1.9 \pm 1.46_a$
	50	5	$(1.7 \pm 1.47)_a$	5	$(5.4 \pm 0.81)_a$	5	$2.7 \pm 1.13_a$
	150	6	$(1.6 \pm 2.37)_a$	6	$(10.1 \pm 2.48)_a$	5	$3.3 \pm 1.43_a$
	450	6	$2.2 \pm 1.00_a$	6	$(7.9 \pm 1.44)_a$	6	$3.5 \pm 0.71_a$
Female	DIMP 0	23	$(2.8 \pm 1.28)_a$	24	$(6.9 \pm 0.74)_a$	24	$5.5 \pm 1.20_a$
	50	22	$(1.6 \pm 0.93)_a$	22	$(4.3 \pm 0.98)_a$	22	$4.0 \pm 1.02_a$
	150	24	$0.0 \pm 1.26_a$	24	$(7.3 \pm 1.23)_a$	24	$5.4 \pm 1.20_a$
	450	23	$(0.1 \pm 0.90)_a$	23	$(6.5 \pm 0.92)_a$	22	$5.5 \pm 0.66_a$
Combined Sexes	DIMP 0	28	$(2.9 \pm 1.16)_a$	29	$(7.4 \pm 0.69)_a$	29	$4.8 \pm 1.05_a$
	50	27	$(1.6 \pm 0.81)_a$	27	$(4.5 \pm 0.82)_a$	27	$3.7 \pm 0.86_a$
	150	30	$(0.3 \pm 1.12)_a$	30	$(7.9 \pm 1.12)_a$	29	$5.0 \pm 1.03_a$
	450	29	$0.4 \pm 0.77_a$	29	$(6.8 \pm 0.79)_a$	28	$5.1 \pm 0.56_a$

Table 51. Effect of chronic administration of DIMP to mink upon feed consumption (g ± S.E.) by date.

Date	DIMP treatment (ppm)			
	0	50	150	450
8/3	$250 \pm 7.3_a$ [1]	$258 \pm 8.7_a$	$268 \pm 6.0_a$	$234 \pm 11.1_a$
8/18	$268 \pm 21.6_a$	$289 \pm 16.9_a$	$309 \pm 18.6_a$	$252 \pm 19.0_a$
9/1	$262 \pm 20.0_a$	$192 \pm 15.5_b$	$212 \pm 15.8_a$	$204 \pm 15.3_a$
9/17	$277 \pm 22.1_a$	$234 \pm 21.8_a$	$268 \pm 19.0_a$	$262 \pm 17.8_a$
9/30	$292 \pm 20.9_a$	$227 \pm 13.4_a$	$260 \pm 13.5_a$	$243 \pm 15.6_a$
10/13	$233 \pm 18.5_a$	$236 \pm 18.4_a$	$237 \pm 16.7_a$	$221 \pm 12.7_a$
11/3	$247 \pm 28.0_a$	$245 \pm 21.2_a$	$279 \pm 14.8_a$	$242 \pm 16.1_a$
11/15	$233 \pm 18.6_a$	$182 \pm 19.4_a$	$231 \pm 14.9_a$	$172 \pm 16.0_b$

[1] Means in the same row with the same subscript are not significantly different from the control ($P > 0.05$).

Table 52. Calculation of estimated daily intake of DIMP by mink fed DIMP at various levels for 12 months.

DIMP level in diet (ppm)	Mean daily feed consumption (g)[1]	DIMP ingested/day (mg)	Mean body wt. (g)[2]	Daily ingested dose (mg/kg/day)
0	258	0	1071	0
50	233	11.65	1061	10.98
150	258	38.70	1043	37.10
450	229	103.05	1084	95.06

[1] Represents mean feed consumption for 8 measurements taken over 4 months.

[2] Represents mean body weight for 18 measurements taken over 12 months.

Table 53. Effect of chronic dietary administration of DIMP to male and female mink upon peripheral blood mean packed cell volume (hematocrit %).

Sex	Treatment	Date measured and number included in analysis							
		7/21/77		10/18/77		1/17/78		6/30/78	
		N	Hct. % ± S.E.	N	Hct. % ± S.E.	N	Hct. % ± S.E.	N	Hct. % ± S.E.
Males	DIMP 0	6	$46.7 \pm 0.84_a$ [1]	5	$52.4 \pm 0.73_a$	5	$53.5 \pm 1.05_a$	5	$52.3 \pm 1.00_a$
	50	6	$47.1 \pm 1.19_a$	5	$55.4 \pm 2.04_a$	5	$55.2 \pm 1.91_a$	5	$56.3 \pm 1.66_a$
	150	6	$44.9 \pm 0.80_a$	6	$52.6 \pm 0.85_a$	6	$52.8 \pm 0.75_a$	6	$55.3 \pm 0.61_b$
	450	6	$46.4 \pm 0.24_a$	6	$55.0 \pm 0.21_a$	6	$57.2 \pm 0.52_a$	6	$57.0 \pm 0.87_c$
Females	DIMP 0	24	$46.7 \pm 0.69_a$	24	$54.0 \pm 0.37_a$	24	$55.0 \pm 0.46_a$	24	$54.3 \pm 0.73_a$
	50	24	$46.2 \pm 0.69_a$	24	$54.5 \pm 0.42_a$	22	$53.9 \pm 0.63_a$	21	$53.7 \pm 0.72_a$
	150	24	$46.4 \pm 0.49_a$	24	$53.5 \pm 0.38_a$	24	$53.8 \pm 0.54_a$	21	$54.0 \pm 0.78_a$
	450	24	$47.2 \pm 0.58_a$	24	$51.8 \pm 1.96_a$	22	$54.7 \pm 0.64_a$	19	$55.4 \pm 0.59_a$
Combined Sexes	DIMP 0	30	$46.7 \pm 0.58_a$	29	$53.7 \pm 0.35_a$	29	$54.7 \pm 0.44_a$	29	$53.9 \pm 0.65_a$
	50	30	$46.4 \pm 0.61_a$	29	$54.7 \pm 0.43_a$	27	$54.1 \pm 0.63_a$	26	$54.2 \pm 0.69_a$
	150	30	$46.1 \pm 0.44_a$	30	$53.3 \pm 0.42_a$	30	$53.6 \pm 0.47_a$	27	$54.3 \pm 0.63_a$
	450	30	$47.1 \pm 0.48_a$	30	$52.5 \pm 1.59_a$	28	$55.3 \pm 0.55_a$	25	$55.8 \pm 0.52_a$

[1] Means in the same column with the same subscript are not significantly different from their respective control values ($P > 0.05$).

Table 54. Effect of chronic dietary administration of DIMP to male and female mink upon peripheral blood hemoglobin concentration.

Sex	Treatment (ppm)	Mean hemoglobin concentration (g/dl ± S.E.) by date and number of mink							
		N	7/21/77	N	10/18/77	N	1/17/78	N	6/30/78
Males	DIMP 0	6	$17.7 \pm 0.27_a$ [1]	5	$19.8 \pm 0.96_a$	5	$20.3 \pm 0.37_a$	5	$19.9 \pm 0.36_a$
	50	6	$17.4 \pm 0.44_a$	5	$22.3 \pm 0.84_a$	5	$19.7 \pm 0.75_a$	5	$21.1 \pm 0.20_a$
	150	6	$17.2 \pm 0.41_a$	6	$19.8 \pm 0.96_a$	6	$20.6 \pm 0.44_a$	6	$21.1 \pm 0.76_a$
	450	6	$17.5 \pm 0.33_a$	6	$21.4 \pm 0.44_a$	6	$21.5 \pm 0.22_a$	6	$21.6 \pm 0.39_a$
Females	DIMP 0	24	$17.6 \pm 0.27_a$	24	$21.9 \pm 0.27_a$	24	$20.3 \pm 0.20_a$	23	$20.0 \pm 0.30_a$
	50	24	$17.4 \pm 0.26_a$	24	$21.8 \pm 0.31_a$	22	$20.0 \pm 0.24_a$	21	$19.0 \pm 1.00_a$
	150	24	$17.9 \pm 0.24_a$	22	$21.7 \pm 0.20_a$	24	$19.7 \pm 0.27_a$	22	$19.9 \pm 0.36_a$
	450	24	$17.9 \pm 0.20_a$	23	$21.0 \pm 0.44_a$	22	$20.1 \pm 0.19_a$	19	$20.0 \pm 0.28_a$
Combined Sexes	DIMP 0	30	$17.6 \pm 0.22_a$	29	$21.5 \pm 0.32_a$	29	$20.3 \pm 0.18_a$	28	$20.0 \pm 0.26_a$
	50	30	$17.4 \pm 0.23_a$	29	$21.9 \pm 0.30_a$	27	$20.0 \pm 0.24_a$	26	$19.4 \pm 0.82_a$
	150	30	$17.8 \pm 0.21_a$	28	$21.3 \pm 0.30_a$	30	$19.9 \pm 0.24_a$	28	$20.2 \pm 0.32_a$
	450	30	$17.8 \pm 0.18_a$	29	$21.1 \pm 0.36_a$	28	$20.4 \pm 0.19_a$	25	$20.4 \pm 0.27_a$

[1] Means in the same column with the same subscript are not significantly different from their respective control values ($P > 0.05$).

Table 55. Effect of chronic dietary administration of DIMP to male and female mink upon peripheral blood mean corpuscular hemoglobin concentration (MCHC).

Sex	Treatment (ppm)	Mean corpuscular hemoglobin concentration (% ± S.E.) by date and number of mink							
		N	7/21/77	N	10/18/77	N	1/17/78	N	6/30/78
Males	DIMP 0	6	$38.0 \pm 0.79_a$ [1]	5	$37.7 \pm 1.72_a$	5	$38.0 \pm 0.95_a$	5	$37.9 \pm 0.72_a$
	50	6	$37.0 \pm 0.43_a$	5	$40.1 \pm 1.00_a$	5	$35.6 \pm 1.17_a$	5	$37.6 \pm 0.60_a$
	150	6	$38.0 \pm 0.71_a$	6	$37.7 \pm 1.84_a$	6	$39.1 \pm 1.07_a$	6	$38.1 \pm 0.80_a$
	450	6	$37.7 \pm 0.93_a$	6	$38.9 \pm 0.95_a$	6	$37.5 \pm 0.32_a$	6	$37.9 \pm 0.46_a$
Females	DIMP 0	24	$37.7 \pm 0.62_a$	24	$40.6 \pm 0.35_a$	24	$37.3 \pm 0.34_a$	23	$36.9 \pm 0.43_a$
	50	24	$37.8 \pm 0.41_a$	24	$40.0 \pm 0.51_a$	22	$37.2 \pm 0.53_a$	21	$37.4 \pm 0.49_a$
	150	24	$38.7 \pm 0.53_a$	22	$40.3 \pm 0.31_a$	24	$36.8 \pm 0.22_a$	21	$37.1 \pm 0.59_a$
	450	24	$37.9 \pm 0.38_a$	23	$39.0 \pm 0.83_a$	22	$36.8 \pm 0.17_a$	19	$36.1 \pm 0.48_a$
Combined Sexes	DIMP 0	30	$37.8 \pm 0.52_a$	29	$40.1 \pm 0.45_a$	29	$37.4 \pm 0.33_a$	28	$37.1 \pm 0.39_a$
	50	30	$37.6 \pm 0.34_a$	29	$40.0 \pm 0.46_a$	27	$36.9 \pm 0.46_a$	26	$37.4 \pm 0.42_a$
	150	30	$38.5 \pm 0.45_a$	28	$39.8 \pm 0.51_a$	30	$37.3 \pm 0.33_a$	27	$37.3 \pm 0.49_a$
	450	30	$37.9 \pm 0.36_a$	29	$39.0 \pm 0.68_a$	28	$36.9 \pm 0.16_a$	25	$36.5 \pm 0.41_a$

[1] Means in the same column with the same subscript are not significantly different from their respective control values ($P > 0.05$)

Table 56. Effect of chronic administration of DIMP to adult mink upon differential leukocyte count.

Treatment (ppm)	N	Leukocyte cell type (% ± S.E.)					
		Basophils	Eosinophils	Band-neutrophils	Segmented neutrophils	Lymphocytes	Monocytes
DIMP 0	27	$0.4 \pm 0.12_a$ [1]	$4.0 \pm 0.52_a$	$1.2 \pm 0.28_a$	$56.8 \pm 2.67_a$	$34.4 \pm 2.42_a$	2.0 ± 0.1
50	25	$0.2 \pm 0.07_a$	$3.7 \pm 0.76_a$	$1.4 \pm 0.30_a$	$57.9 \pm 2.73_a$	$34.7 \pm 2.50_a$	1.9 ± 0.2
150	26	$0.2 \pm 0.10_a$	$3.3 \pm 0.47_a$	$1.5 \pm 0.33_a$	$62.7 \pm 1.91_a$	$29.9 \pm 2.06_a$	1.9 ± 0.2
450	26	$0.3 \pm 0.10_a$	$4.3 \pm 0.49_a$	$1.5 \pm 0.32_a$	$56.1 \pm 3.36_a$	$35.5 \pm 3.08_a$	2.0 ± 0.2

[1] Means with the same subscript are not significantly different from the control ($P > 0.05$).

gestation length, fecundity, kit weight at birth, or secondary sex ratios were noted for the DIMP-treated animals. Kit weight at birth was significantly greater for 50 ppm DIMP-treated animals than controls. Male fertility, as estimated by presence of sperm in post-coital vaginal aspirations, was not adversely affected by chronic DIMP administration.

Whelping dam and kit performance during lactation was not significantly different for DIMP-treatment groups with respect to the controls (Table 58). No significant differences were found in kit mortality, kit weight at 4 weeks of age, or body weight of lactating females at 4 weeks post-partum.

Gross and histological examination at the termination of the test revealed no consistent pathological changes for any DIMP treatment group. Organ weights were not significantly different for DIMP-treated animals with respect to controls (Table 59).

Discussion

The chronic ingestion of DIMP by mink was associated with a higher mortality for DIMP-treated animals than for controls. As previously indicated in this report, chronic ingestion of DIMP by Mallard ducks caused no excessive mortality in birds fed diets that contained as high as 10,000 ppm DIMP. Likewise, DIMP chronically administered in the diet to Bobwhite quail at levels up to 1200 ppm failed to increase mortality above that of controls. In a study designed to test the effects of DIMP upon reproductive performance in rats, chronic ingestion of DIMP at 10 or 1000 ppm in the drinking water for 13 and 19 weeks (males and females, respectively) caused no increase in mortality to this species (Hardesty *et al.*, 1977).

Natural mortality for first-year mink in a commercial fur ranch operation approaches six percent annually (Kennedy, 1952). Since the mortality associated with chronic DIMP administration was greater than this natural mortality, preliminary evidence exists for a chronic toxic effect of DIMP ingestions to mink (especially females), at moderately high doses.

The body weight changes which resulted in mink on either the control or the DIMP-treated diets are in agreement with the growth of mink reported elsewhere (Aulerich and Schaible, 1965; Kumeno, *et al.*, 1970; Oldfield *et al.*, 1968; Seier *et al.*, 1970; Travis and Schaible, 1961).

Feed consumption, was not differentially affected in a trend consistent with dose. Sporadic differences in the consumption of test diets, as compared to the control diet, suggested no demonstrable pattern of differences in palatability, and were most likely attributable to chance or sampling error.

Since ingestion of approximately one-fifth of the calculated LD_{50} by mink on the 450 ppm diet caused no growth impairment or

Table 57. Effect of DIMP on reproductive performance of mink.

	DIMP treatment (ppm)			
	0	50	150	450
No.♀ mated	24	22	22	22
Avg. no. times mated	2.0	1.9	1.9	1.9
% ♀ whelped	62.5	54.5	77.2	68.2
Avg. length of gestation (days ± S.E.)	$49.7 \pm 1.39_a$ [1]	$51.5 \pm 1.93_a$	$50.5 \pm 1.41_a$	$51.5 \pm 0.70_a$
No. of kits at birth: Alive	74	43	96	77
Dead	12	4	9	5
No. live kits/ ♀ whelped ± S.E.	$4.9 \pm 0.41_a$	$3.6 \pm 0.51_a$	$5.7 \pm 0.51_a$	$5.1 \pm 0.56_a$
Avg. wt. of kits at birth (g ± S.E.)	$9.3 \pm 0.32_a$	$10.9 \pm 0.42_c$	$9.4 \pm 0.24_a$	$9.3 \pm 0.34_a$
Secondary ex ratio, no. ♂ kits/ no. ♀ kits	0.95	1.86	1.00	1.08

[1] Means in the same row with the same subscript are not significantly different from the control ($P > 0.05$).

Table 58. Performance of suckling offspring and dams fed DIMP.

	DIMP treatment (ppm)			
	0	50	150	450
Whelping ♀'s lactating at 4 wks. (%)	93	92	94	93
Kit mortality to 4 wks. (%)	20.3	20.9	20.8	19.5
No. kits/♀ lactating ± S.E.	$4.2 \pm 0.46_a$ [2]	$3.1 \pm 0.44_a$	$4.8 \pm 0.53_a$	$4.4 \pm 0.63_a$
Avg. wt. of kits at 4 wks. (g ± S.E.)	$157.6 \pm 4.3_a$	$155.7 \pm 5.5_a$	$145.7 \pm 3.8_a$	$144.1 \pm 4.8_a$
Kit biomass[1]	663.5	481.1	692.1	638.4
Avg. wt. of whelping dams (g ± S.E.)	$994 \pm 33.8_a$	$985 \pm 34.8_a$	$997 \pm 24.5_a$	$964 \pm 23.1_a$
Avg. wt. of lactating ♀'s 4 wks. post partum (g ± S.E.)	$912 \pm 28.6_a$	$872 \pm 44.6_a$	$894 \pm 29.7_a$	$838 \pm 19.9_a$

[1] Biomass = average kit body weight gain between birth and 4 weeks of age x the average number of kits raised per lactating female.

[2] Means in the same row with the same subscript are not significantly different from the control ($P > 0.05$).

Table 59. Effect of chronic administration of DIMP to mink on organ weights (g ± S.E.) at necropsy.

Organ	DIMP treatment (ppm) 0	50	150	450
Liver	25 ± 1.1$_a$ [1]	25 ± 1.4$_a$	24 ± 1.0$_a$	25 ± 1.1$_a$
Spleen	2.7 ± 0.24$_a$	3.0 ± 0.29$_a$	2.9 ± 0.20$_a$	3.2 ± 0.24$_a$
Kidney	4.7 ± 0.22$_a$	4.6 ± 0.22$_a$	4.6 ± 0.21$_a$	4.7 ± 0.21$_a$
Lungs	7.8 ± 0.55$_a$	7.4 ± 0.42$_a$	8.0 ± 0.45$_a$	8.4 ± 0.47$_a$
Adrenals	0.10 ± 0.009$_a$	0.08 ± 0.008$_a$	0.10 ± 0.008$_a$	0.09 ± 0.007$_a$
Heart	5.8 ± 0.29$_a$	5.8 ± 0.30$_a$	6.2 ± 0.38$_a$	5.9 ± 0.31$_a$
Gonads:				
Testes	1.5 ± 0.1$_a$	1.5 ± 0.2$_a$	1.2 ± 0.1$_a$	1.7 ± 0.2$_a$
Ovaries	0.15 ± 0.01$_a$	0.13 ± 0.01$_a$	0.14 ± 0.01$_a$	0.12 ± 0.01$_a$
Brain	7.9 ± 0.17$_a$	8.2 ± 0.15$_a$	8.1 ± 0.21$_a$	8.0 ± 0.19$_a$

[1] Means in the same row with the same subscript are not significantly different from the control ($P > 0.05$).

radical change in appetite, it is unlikely that metabolic efficiency of food conversion was significantly altered by this chemical, at the concentrations used.

Hematological parameters were not appreciably different in value from those reported by other workers. Hematocrit values (packed cell volume) similar to control values were reported by Asher et al. (1976), Fletch and Karstad (1972), Kubin and Mason (1948), and Rotenberg and Jorgensen (1971). Hemoconcentration was reported as a normal occurrence in ranch mink during the winter months and was attributed to decreased water consumption (Asher et al., 1976; Skrede, 1970). The increase in hematocrit values recorded at the termination of this test for males on the 150 and 450 ppm DIMP diets may have been related to a decrease in water intake with resultant hemoconcentration.

Hemoglobin values and mean corpuscular hemoglobin concentrations were in good agreement with values published elsewhere (Fletch and Karstad, 1972; Kubin and Mason, 1948).

Differential leukocyte counts of blood taken from mink at the termination of the chronic test differed slightly from counts made by Fletch and Karstad (1972). These workers showed approximately equal percentages of mature (e.g. segmented) neutrophils and lymphocytes (43% each), and an appreciably greater number of monocytes (9%) than found in the animals in this study. However, Asher et al. (1976) have shown a seasonal and age dependent variation in white cell percentages in mink. Mature (segmented) neutrophils were shown to comprise as high as 75% of all leukocytes during the reproductive season, with lymphocytes comprising as little as 15% during the same period. Monocytes were also shown to undergo seasonal shifts, but in concurrence with Fletch and Karstad (1972), monocytes remained in the 6-8% range throughout the year. Except for the depressed numbers of monocytes, the overall leukocyte percentages found in mink at the termination of this study are well correlated with values for that time of the year reported by Asher et al. (1976). Both Gilbert (1969) and Kennedy (1935) reported monocyte numbers in the 1-2% range in adult mink, but as in the counts recorded by Fletch and Karstad (1972), neutrophils and lymphocytes were nearly equally represented. Hence, the lower monocyte numbers reported in this study are in concurrence with two previous studies, whereas the values obtained for the remaining leucocyte types are in agreement with a number of other previously completed studies.

DIMP was not shown to seriously alter the reproductive capacity of mink when chronically ingested. DIMP chronically administered to Mallard ducks and to Bobwhite quail did not have any adverse effect upon fertility, hatchability, eggshell thickness, or hatchling survival at dietary levels of 10,000 ppm and 1200 ppm, respectively. However, egg production in both species was reduced at these dietary levels. Hardesty et al. (1977) failed to demonstrate any chronic adverse effect upon reproduction in rats given 10 or 1000 ppm DIMP in their drinking water for 13 and 19 weeks (males and females, respectively). The increase in kit weight and aberrant secondary

sex ratio observed on the 50 ppm DIMP diet was probably associated with chance variation and/or sampling error, since a similar effect was not recorded at higher doses. Other reproductive indices (spermatogenesis, gestation length, whelping rate, litter size, and number of stillborn kits) were paralleled by data reported in other studies (Aulerich et al., 1963; Aulerich and Ringer, 1977; Ender, 1952; Hansson, 1947; Schaible and Travis, 1958).

Performance of mink kits for DIMP-treatment groups was likewise unaffected by chronic ingestion of DIMP by lactating females. Kit mortality and growth data for all groups were similarly in agreement with data reported by Aulerich et al. (1975), Aulerich and Ringer (1977), and Oldfield et al. (1968).

At the termination of the experiment no gross or histopathological abnormalities were found to be consistent with any particular DIMP treatment. Organ weights were not appreciably different from weights given in other studies (Aulerich and Ringer, 1977; Wood et al., 1965). Kidney and lung weights for mink in this study were slightly lighter than the weights reported for those organs by Wood et al. (1965). Conversely heart weights of mink in this study were found to be greater than reported by Wood et al. (1965). The lethal agent used in terminating animals was shown to affect the individual organ weights by these same workers. Since the method employed in this study to terminate the animals (cervical dislocation) was different from that employed by Wood and co-workers (electrocution), the differences found in comparison of organ weights may be due to the different euthanatization techniques.

CONCLUSIONS

1. The acute oral LD_{50} of DIMP for mink was 503 mg/kg BW with a 95% confidence interval of 379-668 mg/kg BW.

2. A 21 day subacute dietary LC_{50} of DIMP for mink was estimated to be greater than 10,000 ppm.

3. Chronic ingestion of dietary DIMP had no effect upon growth, reproductive success or neonate performance. A slightly higher mortality occurred in females fed all DIMP treatments than those fed the control diet.

TISSUE RESIDUES IN BOBWHITE QUAIL AND MALLARD DUCKS FED OR DOSED ^{14}C - DIISOPROPYL METHYLPHOSPHONATE

INTRODUCTION

The future restoration of military installations previously exposed to pollutants requires information on the biological hazards of these pollutants. One possible hazard to consider is that wildlife, including birds, would become vectors in passing the pollutants along the food chain to their predators. Thus, consideration should be given to the possibility that the pollutants are not only a hazard to the animals being exposed, but also those preying on the exposed animals.

Birds are particularly difficult to keep out of military reservations because peripheral fencing does not limit their boundary. Insects and/or plants, as well as water on the premises may serve as reservoirs for pollutants. Consumption of these pollutants could result in tissue residues. A chemical of concern on some reservations is diisopropyl methylphosphonate (DIMP). To assess the possible residue levels this may induce in two species of birds, the Bobwhite quail, Colinus virginianus, and the Mallard duck, Anas platyrhynchos, were fed or dosed ^{14}C labeled DIMP. The rate of accumulation and depletion of the radioactivity were ascertained. Presumably, this information would reveal the body burden to short exposure of these pollutants, and the rapidity for depletion of residues upon release from such exposure.

METHODS AND PROCEDURES

Feeding

The experiments were conducted in room #1 of Building #4 on the Michigan State University's Poultry Science Research and Teaching Center (PSRTC). Two adult species of birds, Bobwhite quail and Mallard ducks, were used in the study. The quail were housed in battery brooders, 6 decks high divided into 2 compartments on each deck. Each compartment was 99.4 x 68.6 x 24.1 cm (length x width x height) with 6 quail, 3 of each sex, in each compartment. The Bobwhite quail were from a colony maintained for research and teaching at the PSRTC.

Mallard ducks originated from two sources, Max McGraw Wildlife Foundation, Dundee, Illinois, 60118, and Frost Game Farm, Colona, Wisconsin, 54930. They were phenotypically indistinguishable from wild Mallards. They were regularly housed in pens measuring 152.4 x 152.4 x 76.2 cm (length x width x height). However, for the experiment the ducks were moved into growing-type batteries, 4 decks high, each deck measuring 121.9 x 76.2 x 33.0 cm (length x width x height). Six ducks, 3 of each sex, comprised a group in a compartment.

Supplemental heat was provided in the room to maintain a temperature of 12.8°C. The experiments involved with the feeding of ^{14}C-DIMP were conducted during February 5 to 24, 1977. There was a 9-day pretest period during which feed intake and body weight were

monitored. This was followed by the experimental period during which radioactive diets were fed and the birds killed according to the schedule in Table 60.

Animal care was in accordance with N.I.H. policy, Public Law, and the guidelines of HEW.

The diet fed to the quail was a stock breeder ration (Table 61) prepared by a local feed mill to specifications issued by the Michigan State University's Department of Poultry Science. The ration fed to the ducks was a commercial ration (unknown formula) specified for breeder ducks. Feed was provided ad libitum.

The radioactive DIMP for the experiments was obtained from New England Nuclear[1], and was checked by them for purity just prior to shipment. DIMP was shown to be greater than 96% pure. It was methyl-^{14}C labeled and the specific activity was 3.05 mCi/mM. At the molecular weight of 180 the specific activity for ^{14}C-DIMP was 16.9 µCi/mg.

The radioactive compound was blended into the feed via a premix. The latter was prepared by grinding 1 kg of the breeder ration to pass through a #20 (U.S. Bureau of Standards) sieve, and then adding a weighed amount of cold chemical previously blended with a weighed amount of the ^{14}C-chemical to yield the calculated dilution and quantity of the chemical to prepare a diet with 100 mg of ^{14}C-DIMP per kg of diet. Actual preparation consisted of 16.5 mg of ^{14}C-DIMP plus 2233.7 mg of non-radioactive DIMP, and 2000 mg of this blended with 998 g of sifted diet which yielded a premix with 2 mg ^{14}C-DIMP/g diet. The final rations containing chemical at 100 ppm (mg/kg) were blended in closed containers by tumbling the premix with diet at 5% of dietary weight.

Dosing Experiments

The procedures for housing the Bobwhite quail and Mallard ducks for the dosing experiments were the same as those used in the feeding experiment. The ducks were dosed, per os, on September 19, 1977 and the quail on September 26, with ^{14}C-DIMP according to the protocol in Table 62. The radioactive compound was administered directly into the crop using polyethylene tubing attached to a syringe. Corn oil was the carrier. The dosing solutions of corn oil with radioactive chemical were prepared by adding stock ^{14}C-DIMP to corn oil containing 5% by weight of the respective chemical. The final solutions of corn oil for dosing contained 0.39 µCi/ml of ^{14}C-DIMP to dose the ducks, 1.76 µCi/ml of ^{14}C-DIMP to dose the quail. The calculated dose to be administered was based on 100 mg of chemical per kg body weight, and a target of about 1 µCi of ^{14}C per bird.

---

[1] The citation of a manufacturer's name does not constitute an endorsement by the Deaprtment of the Army.

TABLE 60. THE PROTOCOL TO DETERMINE THE DISTRIBUTION OF ^{14}C FROM ^{14}C-LABELED DIMP IN TWO SPECIES OF BIRDS (BOBWHITE QUAIL AND MALLARD DUCK) GIVEN THE RADIOLABELED COMPOUND IN THE DIET, AND THE PATTERN FOR DEPLETION OF ^{14}C AFTER WITHDRAWAL OF THE RADIOACTIVE DIET AND SUBSTITUTION OF FEED WITHOUT THE ABOVE CHEMICAL.

		Number of birds sacrificed at stated time[1]						
		Controls killed		Days fed ^{14}C-chemical		Days after withdrawal		
Species	Sex	Day 0	Day 10	Day 3	Day 5[2]	Day 3	Day 6	Σ
Bobwhite quail	♀	3	3	3	3	3	3	18
	♂	3	3	3	3	3	3	18
		6	6	6	6	6	6	36
Mallard duck	♀	3	3	3	3	3	3	18
	♂	3	3	3	3	3	3	18
		6	6	6	6	6	6	36

[1] Samples to be processed for radioactivity: red blood cells, plasma, liver, muscle, kidney, skin, brain, adipose.

[2] Day 5 on feed containing chemical is day zero of withdrawal.

TABLE 61. THE COMPOSITION OF THE DIET FED TO QUAIL IN THE FEEDING EXPERIMENTS WITH ^{14}C-DIMP

Ingredient	Amount per 1000 parts
Corn, #2 yellow	450.2
Soybean meal, 49%	327
Meat scrap, 50%	50
Alfalfa meal, dehy.	45
Animal fat, stabl.[1]	57
Limestone	50
Dicalcium phosphate	7
Choline chloride, 50%	3
Methionine hydroxy analogue	1
Salt, iodized	3.8
Mineral mix A	3
Vitamin mix A	3

[1] Ethoxyquin (Antioxidant) 56.8 mg/kg

The birds were fasted overnight prior to receiving the single oral dose of radioactive compound in corn oil.

Killing and Tissue Harvesting

The procedure for procuring tissue samples was common to both the feeding and dosing experiments. Prior to being killed, a blood sample of 3 to 5 ml was obtained from a duck or quail by cardiac puncture using a heparinized syringe and stainless steel needle, 3.81 cm x 20 gauge and 2.54 cm x 22 gauge, for duck and quail, respectively. Ducks were killed by cervical dislocation; the quail were killed either with an overdose of chloroform in a closed container, or by cervical dislocation. The blood was processed immediately to obtain hematocrit values, and the remaining greater portion was transferred to chilled test tubes set in an ice bath. The blood was brought from the PSRTC to the laboratory for centrifugation and the plasma separated from the red blood cells (rbcs). The latter were washed twice with 3 ml of 0.9% saline. Then plasma and rbcs were frozen at -21°C, and stored in this state until thawed for ^{14}C analysis. Samples of tissues from breast muscle, skin (without feathers), adipose from the abdominal area, kidneys, liver, and brain were immediately procured from the dead bird, wrapped in individual plastic bags with identification, and stored on ice until brought into the laboratory. Then they were transferred to a freezer at -21°C and stored in this state until analyzed.

Preparation of Tissues for ^{14}C Counting, and Counting Methodology

Plasma samples were thawed and 200 µl pipetted into vials for liquid scintillation counting. Twelve ml of 3a70ß "Complete Counting Cocktail" (Research Products Int'l. Corp. Elk Grove Village, IL, 60007) were added to the vial, shaken vigorously to disperse the plasma, and then counted for ^{14}C. RBCs were thawed and stirred with a stainless steel spatula to effect uniform distribution of sample. A sample of rbcs was accurately weighed to within $\pm$ 1 mg of 100 mg in a tared vial for liquid scintillation counting by drop-wise addition of rbcs from the spatula. To this was added 1 ml of Unisol^{TM}, a tissue solubilizer. The sample was heated at 50°C for 3 hours in an oven, and/or allowed to stand overnight to solubilize the sample. Sometimes 48 hours of solubilization were required for complete preparation of the sample. Then 10 ml of Unisol^{TM} complement were added to the vial, followed by 2-4 drops of 30% hydrogen peroxide to reduce coloration. The vial cap was put on tightly and the vial shaken. Then the cap was unscrewed and the vial permitted to stand for 20 minutes. The cap was returned onto the vial and the vial counted for ^{14}C.

Samples from the other tissues were obtained by cutting chunks into smaller and smaller pieces, and then randomly selecting tiny pieces to obtain an accurately weighed amount to within $\pm$ 1 mg of 100 mg in a tared vial. These samples were solubilized with 1 ml Unisol^{TM}, as indicated above. The Unisol^{TM} complement was added,

TABLE 62. THE PROTOCOL TO DETERMINE THE DISTRIBUTION AND DEPLETION PATTERN OF ^{14}C-DIMP IN ADULT BOBWHITE QUAIL AND MALLARD DUCKS AFTER A SINGLE ORAL DOSE

Species	Sex	Number of birds killed at stated time to obtain samples[1] for ^{14}C determination				
		Stated time birds were killed--hours				
		0	2	24	48	Σ
Bobwhite quail	♀	3	3	3	3	12
	♂	3	3	3	3	12
		6	6	6	6	24
Mallard ducks	♀	3	3	3	3	12
	♂	3	3	3	3	12
		6	6	6	6	24

[1] Samples to be processed for radioactivity: red blood cells, plasma liver, muscle, kidney, skin, brain, adipose and excreta.

and only on liver samples, which were highly colored, were 2-4 drops of 30% hydrogen peroxide used to reduce coloration.

Samples were counted in either a Nuclear-Chicago Liquid Scintillation Counter Model 724 System; or a Nuclear-Chicago Isocap 300 Series Counter.

A duplicate set of samples of each tissue, representative of all birds on a particular experiment, were processed before they were counted as a set. Each sample was counted for 10 minutes in a complete cycle, and all samples went through 3 cycles. Thus, total count was for 30 minutes. For example, all of the muscle samples from the quail fed ^{14}C-DIMP were removed from the freezer and prepared as one set. These were counted along with ^{14}C-DIMP standard and blank, and a ^{14}C-benzoic acid standard in toluene and a toluene blank. The latter two samples were obtained from Nuclear-Chicago Corp. to be used to establish counting efficiency of standards. A set included, in the case of the feeding experiment, samples obtained from the two groups of controls, one group of 6 quail killed at the start of the experiment, the other group of 6 at the completion of the experiment; and the muscle samples obtained from quail killed on 3 and 5 days of feeding diets with ^{14}C-DIMP, and 3 and 5 days after withdrawal of the radioactive diet.

Calculations for Radioactivity in Tissues

The data as counts per minute (cpm) were analyzed statistically in a minicomputer's program for analysis of variance (ANOV). If significant differences among group means were detected, then the samples from the control groups were compared in the ANOV program. A non-significant F-value indicated that ^{14}C dust from the radioactive diets was not a contributing factor to ^{14}C counts in tissue samples. Therefore, the data from the two control groups were pooled and considered as one group of 12 control samples. The mean value of the control group was subtracted from each cpm of the other individual values to derive a net count for that experimental sample indicative of ^{14}C chemical from the feed. Only one set of control values of the 8 tissues undergoing analyses showed a significant difference indicative of possible ^{14}C dust contamination. However, since none of the other tissues from these control birds showed a comparable effect, we considered the difference of 1.3 cpm to be an aberrant trend. The controls in this case were also pooled to arrive at a mean value for the 12 samples.

Samples were corrected for quenching using internal standards, and for machine efficiency using the ^{14}C-benzoic acid standard supplied by the manufacturer of the scintillation counter. Internal standard corrections varied with each tissue with the greatest quenching occurring in samples prepared from rbcs. Machine efficiency for ^{14}C counting ranged between 72 and 82%, depending upon which scintillation counter was used. The Isocap 300 had the best efficiency.

Detection limits were based on the specified specific activity established by the manufacturer of the ^{14}C-DIMP and the eventual dilution factors in admixing "cold" and radioactive compound for the feeding and dosing experiments. ^{14}C-DIMP was supplied at 3.05 mCi/mM transformable to 16.9 µCi/mg for ^{14}C-DIMP. To determine the detection limit for a particular tissue undergoing radiometric measurements the statistical concept employed was ANOV and Dunnett's t-test for a one-way comparison at a probability value of P = .05 for a significant difference. The standard deviation was that associated with the 12 control values, unless the ANOV showed no significant difference in a comparison of control vs. values from birds receiving radioactive feed or solutions; in those cases, the standard deviation was derived from the Error term of the ANOV. The formula used for calculating the Dunnett's allowance value, A, (Dunnett, 1955) was as follows:

$$A = \text{Dunnett's } t_{.05} \times \text{std. dev.} \times \sqrt{1/n_1 + 1/n_2}$$ where:

(a) Dunnett's t value is obtained from the table at d.f. = 30, and for 4 treatments
(b) Std. dev. is the standard deviation for the 12 control values
(c) n_1 = number of values in the control group
(d) n_2 = number of experimental values in the comparison to the control values

For example: plasma samples of 200 µl counted from Bobwhite quail, each counted for 3 x 10 min. averaged 30.0 $\pm$ 1.37 (mean $\pm$ S.D.) as the background.

Where $A = 2.25 \times 1.37 \times \sqrt{1/12 + 1/1}$ in a comparison of the 12 control values to any 1 experimental value.

A = 3.2 cpm above background would be a significantly (P = 0.05) higher number. Thus, a count of 33.2 (30 + 3.2) would indicate detectable radioactivity.

The detection limits are then calculated by transposing the allowance value from cpm to dpm and dividing by a specific activity of the radioactive compound.

In the case of plasma samples reviewed above, the calculations showed the following:

$$\text{Detection limit} = \text{Allowance value} \times \frac{1}{\text{quench factor}} \times \frac{1}{\text{sample size}} \times \frac{1}{\text{machine efficiency}} \times \frac{1}{\text{specific activity in dpm/µgm}} = \frac{\text{µgm}}{\text{g or ml}}$$

$$= 3.2 \text{ cpm} \times \frac{1}{0.925} \times \frac{1}{0.2 \text{ ml}} \times \frac{1}{0.74} = 23.3 \text{ dpm/ml plasma}$$

The specific activity of ^{14}C-DIMP was 3.05 mCi/mM which is equal to 16.94 μCi/mg. A quantity of 16.5 mg of "radioactive" ^{14}C-DIMP at 16.9 μCi/mg was diluted to a final weight of 2250 mg DIMP, using non-radioactive DIMP. Therefore, a total of 279.5 μCi was diluted to 2250 mg or to a concentration of 0.1242 μCi/mg. At 2.2×10^6 dpm per μCi, this yielded a radioactive compound with 2.2×10^6 dpm $\times$ 0.1242 μCi/mg = 0.2734×10^6 dpm/mg = $\frac{273.4 \times 10^3 \text{ dpm}}{\text{mg}} = \frac{273.4 \text{ dpm}}{\mu\text{g } ^{14}\text{C-DIMP}}$

The detection limit of 23.3 dpm of ^{14}C-DIMP is equivalent to 23.3 dpm $\times \frac{1}{273.4/\text{dpm}/\mu\text{g}} = 0.0852$ μg ^{14}C-DIMP/ml.

The calculations for the detection limits of other tissues followed the above procedure, but with the proper values substituted in each case. These detection limits are listed in each table giving the values of radioactive compound(s) found in the tissues.

Extraction of Feed for Radioactivity

At the conclusion of the feeding experiments involving ^{14}C-DIMP to ducks and quail, samples of the feed were removed, stored in plastic bags and frozen at -21°C. About 6 months afterward they were moved into a refrigerator at 8°C and stored there for 3 months. At that time 2 g samples of the feed were weighed into 50 ml glass centrifuge tubes, and extracted 3 times with 10 ml of either dioxane or chloroform:petroleum ether (1:1) or ethyl acetate, to remove DIMP from feeds with ^{14}C-DIMP. Total volume of extracts was determined, and aliquots of 0.5 ml counted in 12 ml of cocktail. Recoveries of ^{14}C from the feed were calculated based on original ^{14}C specific activity introduced into the feed. One-half gram residue samples of feed remaining in the test tubes after extractions were also counted, and the residue portion weighed to determine the proportion of sample that was counted. Total dpm recovered from extracts and residues after extractions represented recovery of ^{14}C in feed. The proportion of ^{14}C in extractions presumably represented initial compound. No chromatograms were developed on the extractions and residue samples to determine percentages of parent compound remaining.

Results

BODY WEIGHT, FEED INTAKE, AND HEMATOCRIT

Feeding Experiments

Bobwhite quail used in the feeding experiments for ^{14}C-DIMP lost weight during the holding period of 9 days. This can be determined from the data in Table 63 by comparison between initial weight and weight on day 0, the date the experiment started. The quail moved into the batteries to be used for the experiment with ^{14}C-DIMP weighed an average of 184 g (Table 63), and lost about 13 g per bird over the next 9 days. During the time the radioactive diets were fed the body weights improved to some extent in most groups.

TABLE 63. BODY WEIGHTS OF BOBWHITE QUAIL FED ^{14}C-DIMP @ 100 PPM OR FEED WITHOUT DIMP, AND HEMATOCRIT AT TIME OF SACRIFICE

Group	Treatment	Bird No.	Sex	Initial wt. on 2/5/77	Change in body weight from initial weight					Hematocrit %
					day 0 on	day 3 on	day 5 on	day 3 off	day 5 off	
1	None	1583	♂	186 g	- 9 g					39.5
		1586	♂	196	-18					37.0
		1587	♂	183	-12					33.0
		1585	♀	144	+ 3					34.0
		1582	♀	208	-22					29.0
		1588	♀	170	- 7					36.0
		Mean (±S.D.)		181(±22)	-10.8(±8.8)					34.8(±3.6)
2	None	1590	♂	195	- 7	- 3	- 2	- 2	+ 8	35.5
		1592	♂	190	- 5	+ 6	+ 8	+ 7	+ 2	23.8
		1594	♂	191	-15	-10	- 9	- 8	-17	40.0
		1589	♀	193	-19	-10	- 9	-10	-16	30.0
		1591	♀	196	-10	- 7	- 8	- 9	-14	35.0
		1584	♀	186	-10	- 2	0	- 3	-14	37.5
		Mean (±S.D.)		192(±4)	-11(±5.2)	-4.3(±6.1)	-3.3(±6.7)	-4.2(±6.4)	-8.5(±10.7)	33.6(±5.8)
3	^{14}C-DIMP in feed @ 100 ppm starting 2/14	1593	♂	184	-12	+ 5	+ 5	+ 5	+ 2	33.3
		1596	♂	177	-19	- 2	0	- 2	- 9	37.0
		1600	♂	177	-15	- 4	- 1	- 4	-10	37.5
		1595	♀	178	-24	+ 6	+ 7	+ 2	- 2	34.0
		1597	♀	160	-20	- 9	- 5	- 7	- 5	37.5
		1599	♀	172	-14	+ 3	+ 1	0	- 1	33.5
		Mean (±S.D.)		176(±5.5)	-17.3(±4.4)	-0.2(±5.8)	1.2(±4.3)	-1.0(±4.3)	-4.2(±4.7)	35.4(±2.1)
4	^{14}C-DIMP in feed @ 100 ppm starting 2/14	1624	♂	176	-20	-11	- 7	- 8		41.5
		1622	♂	196	-16	-12	- 9	-10		41.0
		1602	♂	174	- 4	+ 8	+10	+ 4		38.0
		1625	♀	179	-16	- 9	- 5	- 3		36.0
		1623	♀	193	-16	- 5	- 5	- 5		39.0
		1603	♀	175	- 8	+ 1	+ 2	- 3		29.0
		Mean (±S.D.)		182(±10)	-13.3(±6.0)	-4.7(±7.8)	-2.3(±7.1)	-4.2(±4.9)		37.4(±4.6)
5	^{14}C-DIMP in feed @ 100 ppm starting 2/14	1607	♂	193	-13	+ 2				41.0
		1601	♂	184	-11	+ 1				39.0
		1604	♂	189	-15	- 7				37.0
		1605	♀	180	- 4	+10				38.0
		1606	♀	169	-12	+ 2				34.0
		1608	♀	201	-23	- 8				36.0
		Mean (±S.D.)		186(±11)	-13(±6.1)	0 (±6.7)				37.5(±2.4)
6	^{14}C-DIMP in feed @ 100 ppm starting 2/14	1689	♂	186	- 9	+ 7	+ 6			39.5
		1690	♂	191	- 9	0	+ 2			39.5
		1693	♂	178	-14	-12	-11			33.5
		1694	♀	180	-11	+ 5	+ 2			40.5
		1692	♀	189	-12	+ 3	- 1			39.5
		1691	♀	194	-16	- 5	- 6			37.0
		Mean (±S.D.)		186(±16)	-11.8(±2.0)	-0.3(±7.1)	-1.3(±6.2)			38.3(±2.6)
										17.0(±4.2)

The controls (Group 2) fared as well as the treated quail. Generally, feed intake was higher during the time the radioactive diets were fed (Table 64), and this appeared to account for the quail regaining some of their body weight.

Calculations reveal that quail fed ^{14}C-DIMP consumed 6.8 mg of the chemical in the first 3 days, or at the rate of 2.27 mg per bird per day. Those fed the diet for 5 days consumed 9.7-10.8 mg (Table 64), or 2.05 mg per bird per day. The total dose of ^{14}C-DIMP on a body weight basis for the 3 and 5 days of feeding was 36 and 55 mg/kg body weight, respectively (Table 64). On a daily basis the average body burden was 11-12 mg/kg body weight.

Hematocrit values for the quail averaged 37 and 35 mg% for males and females, respectively, in the experiment involving DIMP (Table 63). The chemical had no effect on the hematocrit values; controls and treated birds had comparable hematocrits.

The Mallard ducks to be fed ^{14}C-DIMP weighed 1308 and 1105 g for males and females, respectively (Table 65). A loss of body weight occurred in all of the ducks during the holding period, and it amounted to 91 g/bird, on the average, or about 7½% of initial body weight. This was about the same magnitude of loss as a percentage of body weight detected during the holding period for the quail. The larger value for the females reflected either the seasonal trend for these birds to deposit migratory fat, or to be actively in egg production.

Table 66 contains the data on feed intake of the ducks during the experiments with ^{14}C-DIMP. No consistent trends for feed intake to be influenced by the 100 ppm level of the chemical in the diet were observed. Birds that consumed greater quantities of diet with the chemical, consumed amounts of diet comparable to this during the withdrawal period when no chemical was in the diet. The ducks fed ^{14}C-DIMP consumed 29-33 mg per kg of body weight for the total 3 or 5 days during which the radioactive chemical was fed (Table 66). The body burden of ^{14}C-DIMP on a daily basis was calculated to be 6.3 mg per kg of body weight.

Hematocrit values for the male and female ducks used in the ^{14}C-DIMP experiment averaged 41.9 and 43.5%, respectively (Table 65). Feeding ^{14}C-DIMP had no effect on hematocrit values (Table 65).

Dosing Experiments

Quail used in the dosing experiments weighed 199 and 190 g for female and male, respectively (Table 67). The dose of ^{14}C-DIMP was targeted at 100 mg per kg body weight, but the actual quantity given amounted to 102.5 mg per kg body weight (Table 67). When these values were compared to the daily body burden of ^{14}C-DIMP received via the consumption of feed, the oral dose was 9 fold greater for ^{14}C-DIMP. Hematocrit values averaged 33.5 and 38.1 ml% for female and male quail, respectively (Table 67). There was a significant

TABLE 64. THE AMOUNT OF FEED AND ^{14}C-DIMP CONSUMED BY BOBWHITE QUAIL FED THE RADIOACTIVE COMPOUND AT 100 PPM IN THE DIET

Group	Feed Intake - g/b/d						^{14}C intake mg/b	Body wt. g	^{14}C-mg per kg body wt.
	Pre-exptl. period	Experimental period Days 0-3 on	Days 3-5 on	Mean	Withdrawal period Days 0-3 off	Days 3-5 off			
1[a]	(6)[b] 17.9	-		-	-	-	0	181	0
2[a]	(6) 18.1	(6) 19.8	(6) 16.6	18.5	(6) 17.4	(6) 14.8	0	192	0
3	(6) 18.3	(6) 21.8	(6) 17.4	20.0	(6) 17.5	(6) 15.0	10.0	176	56.9
4	(6) 17.8	(6) 20.5	(6) 17.9	19.5	(6) 16.2	-	9.7	182	53.5
5	(6) 17.9	(6) 22.5	-	22.5	-	-	6.8	186	36.3
6	(6) 18.3	(6) 22.7	(6) 20.0	21.6	-	-	10.8	186	58.1

[a] Controls

[b] Number of birds

TABLE 65. BODY WEIGHTS AND HEMATOCRITS OF MALLARD DUCKS FED ^{14}C-DIMP @ 100 PPM OR FEED WITHOUT DIMP

Group	Treatment	Bird No.	Sex	Initial wt. on 2/5/77	Change in body weight from initial weight					Hematocrit %
					day 0 on	day 3 on	day 5 on	day 3 off	day 5 off	
1	None	4817	♂	1322	- 81					40.0
		4819	♂	1318	-195					41.3
		4821	♂	1246	-101					36.5
		4818	♀	1009	- 67					44.8
		4820	♀	946	- 75					44.3
		2622	♀	1067	-130					45.5
		Mean (±S.D.)		1151(±165)	-108(±48)					42.0(±3.4)
2	None	901	♂	1193		+ 44	+ 89	+109	+124	44.5
		6477	♂	1258		-130	-165	-159	-160	40.8
		6475	♂	1228		-203	- 67	- 46	- 15	39.5
		6731	♀	1134		-127	- 71	-103	-128	41.3
		6793	♀	1059		- 30	- 24	- 39	- 56	43.8
		6792	♀	1172		- 89	- 27	0	+ 13	42.8
		Mean (±S.D.)		1174(±71)		-91(±85)	-44(±83)	-40(±92)	-37(±102)	42.1(±1.9)
3	^{14}C-DIMP in feed @ 100 ppm starting 2/16	4713	♂	1346	- 52	- 47	- 20	- 58	- 68	43.8
		904	♂	1328	- 32	-151	-138	-171	-165	41.8
		905	♂	1328	- 32	-136	-169	-216	-244	42.0
		6733	♀	1146	- 58	- 94	- 61	-104	-129	40.8
		6732	♀	1302	- 62	-200	-113	-156	-180	45.5
		995	♀	1343	- 88	- 56	+ 61	- 53	+ 14	41.0
		Mean (±S.D.)		1298(±76)	-54(±21)	-114(±59)	-73(±105)	-126(±65)	-129(±91)	42.4(±1.8)
4	^{14}C-DIMP in feed @ 100 ppm starting 2/14	4896	♂	1398	-100	- 75	- 80	- 67		44.8
		4889	♂	1343	-112	-218	-255	-164		38.3
		4887	♂	1677	-178	-195	-205	-200		44.3
		4888	♀	1028	- 30	- 31	+ 10	+ 28		46.3
		4886	♀	1026	- 99	- 76	- 69	- 63		43.8
		4885	♀	1000	- 52	- 48	- 6	+ 3		43.3
		Mean (±S.D.)		1245(±274)	-95(±51)	-107(±79)	-100(±107)	-77(±90)		43.5(±2.7)
5	^{14}C-DIMP in feed @ 100 ppm starting 2/14	4806	♂	1181	-103	- 41				43.8
		4808	♂	1466	-169	-118				42.6
		4810	♂	1240	- 85	+ 2				41.3
		4809	♀	1047	- 85	- 30				44.6
		4805	♀	1206	-129	- 97				41.1
		4807	♀	1138	-121	-101				44.0
		Mean (±S.D.)		1226(±143)	-115(±32)	-64(±48)				42.9(±1.5)
6	^{14}C-DIMP in feed @ 100 ppm starting 2/14	4812	♂	1245	- 99	- 38	- 48			44.0
		4814	♂	1371	-131	-113	-115			42.3
		4816	♂	1062	- 55	- 4	- 15			42.8
		4811	♀	1169	- 92	- 88	- 98			44.8
		4813	♀	998	- 80	- 67	- 71			42.3
		4815	♀	1029	- 54	0	+ 4			5
		Mean (±S.D.)		1145(±143)	-85(±29)	-52(±46)	-57(±46)			43. .0)

TABLE 66. THE AMOUNT OF FEED AND ^{14}C-DIMP CONSUMED BY MALLARD DUCKS FED THE RADIOACTIVE COMPOUND AT 100 PPM IN THE DIET

Group	Feed Intake - g/b/d						^{14}C intake mg/b	Body wt. g	^{14}C-mg per kg body wt.
	Pre-exptl. period	Experimental period Days 0-3 on	Days 3-5 on	Mean	Withdrawal period Days 0-3 off	Days 3-5 off			
1[a]	(6)[b] 63.9	-	-	-	-	-	0	1151	0
2[a]	(6) 64.6	(6) 48.7	(6) 62.1	54.1	(6) 59.3	(6) 70.8	0	1174	0
3	(6) 61.2	(6) 44.8	(6) 77.1	57.7	(6) 62.3	(6) 59.6	28.9	1298	22.2
4	(6) 64.5	(6) 74.5	(6) 64.1	70.3	(6) 86.5	-	35.2	1245	28.2
5	(6) 64.1	(6)102.9	-	102.9	-	-	30.9	1226	25.2
6	(6) 64.5	(6) 97.3	(6) 43.3	75.7	-	-	37.9	1145	33.1

[a] Controls

[b] Number of birds

TABLE 67. BODY WEIGHT, HEMATOCRIT AND AMOUNT OF RADIOACTIVE CHEMICAL GIVEN TO BOBWHITE QUAIL DOSED ORALLY WITH ^{14}C-DIMP AT 100 MG PER KG BODY WEIGHT

Group	Band No.	Sex	Body wt.-g	Dose	Body wt. mg/kg	Compound given	Time of killing	Hematocrit %
1	2447	♀	205	None	0	None	0 hr.	31.8
	2449	♀	180	"	0	"	"	31.8
	2448	♀	210	"	0	"	"	35.5
	2430	♀	213	"	0	"	"	34.2
	2438	♀	199	"	0	"	"	32.5
	2429	♀	210	"	0	"	"	27.5
	2451	♂	212	"	0	"	"	38.8
	2450	♂	174	"	0	"	"	38.8
	2452	♂	182	"	0	"	"	30.5
	2433	♂	187	"	0	"	"	36.5
	2431	♂	208	"	0	"	"	35.0
	2432	♂	177	"	0	"	"	35.0
			196(±15)					34.7(±3.4)
2	2468	♀	196	20	102	DIMP	2 hr.	30.0
	2466	♀	176	18	102	"	"	36.0
	2465	♀	204	20	98	"	"	39.8
	2467	♂	219	21	95	"	"	41.5
	2469	♂	195	20	102	"	"	46.3
	2470	♂	176	18	102	"	"	39.0
			194(±17)	19.5(±1.2)	100(±3)			38.8(±5.5)
3	2434	♀	204	20	98	DIMP	24 hr.	38.0
	2435	♀	203	20	98	"	"	31.0
	2436	♀	187	20	106	"	"	35.0
	2440	♂	191	20	104	"	"	43.3
	2437	♂	170	19	111	"	"	35.3
	2430	♂	200	20	100	"	"	30.0
			193(±13)	19.8(±0.4)	103(±5)			36.8(±4.1)
4	2441	♀	205	21	102	DIMP	48 hr.	32.3
	NB	♀	187	20	106	"	"	31.3
	2443	♀	205	20	97	"	"	36.3
	2445	♂	194	20	103	"	"	33.0
	2446	♂	160	18	112	"	"	34.5
	2444	♂	206	20	97	"	"	38.3
			192(±18)	19.8(±1.0)	102(±6)			34.3(±2.6)
	Avg	♀	199(±11)					33.5(±3.3)
	Avg	♂	190(±17)					38.1(±3.5)

($P < .01$) difference between these values. Control quail had an average hematocrit of 34.7 ml%, as compared to values in quail dosed with ^{14}C-DIMP of 38.8, 36.8, or 34.3 killed at 2, 24, or 48 hours following the dose. There was no significant ($P > .05$) treatment effect.

The ducks used for the ^{14}C-DIMP dosing experiment had an average body weight of 1128 and 1251 g for female and male ducks, respectively (Table 68). The dose of each chemical was targeted for 100 mg per kg of body weight and the dose was on target (Table 68). The oral dose was 15.9 fold greater than the body burden of ^{14}C-DIMP received from consuming the diets with 100 ppm of the chemical. Hematocrit values for these ducks were 41.1 and 42.2 ml% for females and males, respectively (Table 68). There was no significant ($P > .05$) treatment effect on hematocrit values from either chemical over the 48-hour period following the single oral dose at 100 mg per kg of body weight.

Tissue Residues

A. In order to compare the residue values in tissue obtained from feeding or dosing ^{14}C-DIMP to quail and ducks, the comparative body burden of these chemicals must be reconsidered. In the following table are the amounts of the ^{14}C-DIMP consumed on a daily basis with the values adjusted for the body weight, in kg, of these birds.

Body burden - mg ^{14}C-DIMP per kg body weight

Route of administration	^{14}C-DIMP	
	Quail	Ducks
A. Fed @ 100 ppm	11.5	6.3
B. Dosed, per os, @ 100 mg/kg body wt.	102	100
B/A	8.9	15.9

One should recall that the above comparison is based upon a single oral dose of a chemical in a solvent which is a natural foodstuff, in this case, corn oil, as compared to the feeding approach which introduces the chemical in a dry state in much smaller quantities per unit time, and with a mixture of feed ingredients that may interfere with or enhance absorption. Therefore, not necessarily may the ^{14}C residue values in tissues be at the same comparative relationship as the "B/A values" in the table above.

Table 69 contains the data on tissue ^{14}C equivalents in 8 tissues from quail and ducks receiving ^{14}C-DIMP either by feed or oral dose. Tables in Appendix J of this report contain the individual values for each tissue of each bird that were used to obtain the data in Table 69.

One of the most striking effects to be noted about the data in Table 69 is that the ^{14}C levels in tissues of ducks and quail at the

TABLE 68. BODY WEIGHT, HEMATOCRIT AND AMOUNT OF RADIOACTIVE CHEMICAL GIVEN TO MALLARD DUCKS DOSED ORALLY WITH ^{14}C-DIMP AT 100 MG PER KG BODY WEIGHT. DUCKS WERE KILLED AT 0, 2, 24, OR 48 HOURS AFTER DOSE.

Group	Band No.	Sex	Body wt.-g	Dose mg	Body wt. mg/kg	Compound given	Time of killing	Hematocrit %
1	6049	♀	1060	None	0	None	0 hr.	42.3
	6004	♀	880	"	0	"	"	41.0
	6005	♀	1220	"	0	"	"	41.8
	6006	♀	1180	"	0	"	"	43.1
	6009	♂	1322	"	0	"	"	38.8
	6097	♂	1105	"	0	"	"	41.0
	6092	♂	1405	"	0	"	"	42.3
	6001	♂	1180	"	0	"	"	40.3
			1169(±161)					41.3(±1.4)
2	6016	♀	1095	110	100	DIMP	2 hr.	41.8
	6017	♀	1300	130	100	"	"	39.8
	6018	♀	1260	125	99	"	"	43.3
	6013	♂	1170	120	103	"	"	45.0
	6014	♂	1265	125	99	"	"	44.5
	6015	♂	1305	136	104	"	"	47.0
			1233(±83)	124(±9)	100(±2)			43.6(±2.5)
3	6045	♀	1235	120	97	DIMP	24 hr.	40.0
	6044	♀	1125	110	98	"	"	42.3
	6043	♀	1175	120	102	"	"	41.8
	6046	♂	1205	130	101	"	"	46.8
	6047	♂	1190	120	101	"	"	40.3
	6048	♂	1095	110	100	"	"	40.5
			1184(±70)	118(±3)	100(±2)			42.0(±2.5)
4	6040	♀	1020	100	98	DIMP	48 hr.	40.0
	6041	♀	1200	120	100	"	"	40.0
	6042	♀	925	90	97	"	"	38.0
	6037	♂	1195	120	100	"	"	40.8
	6038	♂	1400	140	100	"	"	41.3
	6039	♂	1420	140	99	"	"	39.5
			1193(±198)	118(±20)	99(±1)			39.8(±1.2)
	Avg	♀	1120(±127)					41.1(±1.5)
	Avg	♂	1251(±115)					42.2(±2.7)

TABLE 69. SUMMARY OF DATA, BASED ON GROUP MEANS, OF ^{14}C ACTIVITY IN TISSUES FROM BOBWHITE QUAIL AND MALLARD DUCKS GIVEN ^{14}C-DIMP VIA THE FEED OR DOSED PER OS.

Tissue ^{14}C-equivalents from ^{14}C-DIMP - µg/g (ppm)

Feed @ 100 ppm in diet			Dose @ 100 mg/kg body wt.			Feed @ 100 ppm in diet			Dose @ 100 mg/kg body wt.		
Sample Time	Quail	Duck	Sample Time	Quail	Duck	Sample Time	Quail	Duck	Sample Time	Quail	Duck
		PLASMA ^{14}C						RBC ^{14}C			
Day 3 on	0.51	0.24	0 hour	0.0	0.0	Day 3 on	0.0	0.11	0 hour	0.0	0.0
Day 5 on	0.14	0.40	2nd hour	154.1	137.9	Day 5 on	0.0	0.13	2nd hour	7.5	5.1
Day 3 off	0.0[a]	0.0	24th hour	0.0	0.0	Day 3 off	0.0	0.0	24th hour	0.0	0.0
Day 5 off	0.0	0.0	48th hour	0.0	0.0	Day 5 off	0.0	0.0	48th hour	0.0	0.0
Detection Limit	0.047	0.011	Detection Limit	0.64	3.99	Detection Limit	0.11	0.061	Detection Limit	0.37	1.84
		LIVER ^{14}C						KIDNEY ^{14}C			
Day 3 on	0.76	0.29	0 hour	0.0	0.0	Day 3 on	1.10	0.32	0 hour	0.0	0.0
Day 5 on	0.32	0.51	2nd hour	115.4	756.3	Day 5 on	0.43	0.51	2nd hour	117.5	180.0
Day 3 off	0.0	0.0	24th hour	0.0	0.0	Day 3 off	0.0	0.0	24th hour	1.26	0.0
Day 5 off	0.0	0.0	48th hour	0.0	0.0	Day 5 off	0.0	0.0	48th hour	0.0	0.0
Detection Limit	0.070	0.033	Detection Limit	0.37	1.07	Detection Limit	0.033	0.033	Detection Limit	0.22	1.35
		ADIPOSE ^{14}C						BRAIN ^{14}C			
Day 3 on	0.05	0.0	0 hour	0.0	0.0	Day 3 on	0.09	0.05	0 hour	0.0	0.0
Day 5 on	0.35	0.0	2nd hour	71.2	15.8	Day 5 on	0.06	0.07	2nd hour	9.31	22.9
Day 3 off	0.0	0.0	24th hour	0.38	0.0	Day 3 off	0.0	0.0	24th hour	0.50	0.0
Day 5 off	0.0	0.0	48th hour	0.0	0.0	Day 5 off	0.0	0.0	48th hour	0.0	0.0
Detection Limit	0.037	0.027	Detection Limit	0.22	0.63	Detection Limit	0.035	0.027	Detection Limit	0.27	0.82
		SKIN ^{14}C						MUSCLE ^{14}C			
Day 3 on	1.11	0.17	0 hour	0.0	0.0	Day 3 on	0.14	0.14	0 hour	0.0	0.0
Day 5 on	0.43	0.16	2nd hour	48.0	45.1	Day 5 on	0.12	0.15	2nd hour	11.1	26.4
Day 3 off	0.12	0.0	24th hour	0.79	0.0	Day 3 off	0.0	0.0	24th hour	0.87	0.0
Day 5 off	0.10	0.0	48th hour	0.90	0.0	Day 5 off	0.0	0.0	48th hour	0.19	0.0
Detection Limit	0.026	0.022	Detection Limit	0.23	0.99	Detection Limit	0.032	0.022	Detection Limit	0.18	1.74

[a] 0.0 = less than detection limit based upon comparison of 12 control vs. 6 exptl. values

2nd hour after the single oral dose were very high. Values from the dosing experiment ranged from a low of 5.1 µg/g for rbcs in ducks, to as high as 756.3 µg/g in the liver of ducks. When these ^{14}C levels in quail and duck tissues taken at the 2nd hour were compared in the table below, four tissues (plasma, skin, rbcs, and adipose) from quail were found to have higher ^{14}C residue levels than the same tissues from ducks; whereas, the other 4 (liver, brain, muscle, and kidney) were high in ducks:

^{14}C-equivalents, µg/g (ppm) in tissues from quail and ducks at the 2nd hour following a single oral dose of ^{14}C-DIMP at 100 mg/kg

	Liver	Plasma	Kidney	Skin	Adipose	Brain	Muscle	RBCs
Quail (Q)	115.4	154.1	117.5	48.0	71.2	9.31	11.1	7.5
Duck (D)	756.3	137.9	180.0	45.1	15.8	22.9	26.4	5.1
D/Q	6.6	0.9	1.5	0.9	0.2	2.4	2.4	0.7

When one compares these ^{14}C residue levels from the dosing experiment to the residues obtained from the feeding experiments the contrast is obvious. Quail and ducks fed ^{14}C-DIMP at 100 ppm had ^{14}C residues predominately less than 1 ppm at day 3 or 5 on radioactive diets (Table 69). Higher values were detected on day 3 than on day 5 in quail, and this appeared to be related to the greater amount of feed consumed on a daily basis during the first 3 days than over the next 2 days (Table 64). If one assumes that the best estimates for ^{14}C values in quail were the higher values obtained on day 3, then compares these values to those obtained on day 5 for ducks, the following comparison is obtained:

^{14}C equivalents, as µg/g (ppm), in quail and duck tissues obtained while being fed ^{14}C-DIMP @ 100 ppm in diet

	Kidney	Skin	Liver	Plasma	Adipose	Muscle	Brain	RBCs
Quail (Q)	1.0	1.11	0.76	0.51	0.85	0.14	0.09	0.0
Duck (D)	0.51	0.16	0.51	0.40	0.027	0.15	0.07	1.13
D/Q	0.5	0.1	0.7	0.8	---	1.1	0.08	---

Four of the tissues (muscle, plasma, brain, and liver) are within 30% of having the same specific values for ^{14}C residues in the two species of birds. The highest values of ^{14}C appeared to accumulate within the organs involved in metabolism and excretion, i.e., liver and kidney. This was also the case for ^{14}C-DIMP levels in the dosing experiments. Adipose, a storage tissue, had 0.85 µg ^{14}C residues/g, in quail on the feeding experiment. Ducks, similarly treated, had no detectable ^{14}C in adipose. The ducks were presumably metabolizing and/or excreting the ^{14}C-DIMP without difficulty when consumed at the rate of 6.3 mg/kg body weight. But when the single oral dose of 100 mg per kg body weight was given, the overwhelming amount of compound resulted in ^{14}C adipose levels of 16 ppm at the 2nd hour of

sampling. Quail dosed with ^{14}C-DIMP also did not retain ^{14}C in adipose tissue (Table 69), and neither quail nor duck from the feeding experiments retained ^{14}C in adipose by day 3 after withdrawal of ^{14}C-DIMP from feed. Thus, DIMP and its metabolites are ordinarily not lipophilic, and thus, not retained in adipose tissues over long periods of time. Its biological half-life in adipose tissue must be extremely rapid in order to clear in 22 hours from 16 ppm to less than 0.6 ppm in ducks, or from 71 ppm to 0.4 ppm in quail (Table 69).

In the feeding trials, ^{14}C residues were less than detection limits by the 3rd day after withdrawal of diets containing ^{14}C-DIMP, except for skin samples from quail. In the latter tissue, ^{14}C residues persisted at 0.1 ppm by day 5 after withdrawal (Table 69). One possible consideration for this unique persistence of ^{14}C in the skin, while other tissues including adipose, had since been depleted of ^{14}C, may be that ^{14}C is incorporated into normal biochemical components of the skin resulting in a non-metabolite residue.

Radioactivity in Stored Feed

Nine months after the feed had been stored, samples were extracted with different soluents to determine recovery values. The results are presented in the following table.

Percent recovery of ^{14}C from diets containing ^{14}C-DIMP

Chemical in Feed	Solvent for Extraction	% ^{14}C Recovered
^{14}C-DIMP	Chloroform:petroleum ether (1:1)	58.4
	Ethyl acetate	42.8
	p-Dioxane	44.5
	DIMP	35.8
	Butanol	60.0

Butanol or chloroform:petroleum ether (1:1) extractions yielded the highest recovery values for ^{14}C-DIMP. The almost 60% recovery of ^{14}C from the feed with DIMP was less than satisfactory but approached a 70-80% range we would have anticipated after a long period of storage. We are unable to explain the unsatisfactory recovery of ^{14}C from the feed with DIMP. Original counting of the corn oil which went into the diet revealed it had the expected amount of ^{14}C-DIMP.

Discussion

DIMP does not belong in the classification of those compounds which persist for long periods of time within the animal's body. Instead, it is rapidly depleted from body tissues of wild-type fowl (Bobwhite quail and Mallard ducks) as evidenced from dosing and feeding experiments. The dosing experiments revealed that despite high levels of ^{14}C residues induced within 2 hours from ^{14}C-DIMP, some as high as 756 ppm, the residue levels were at or below detection limits of 0.3 to 1.0 ppm in 48 hours. Adipose tissue, known

to be a reservoir for certain pesticides and environmental contaminants, did not show the persistence to retain ^{14}C. Based upon these data, one can conclude that the parent compound and/or its metabolit are not particularly lipophilic upon entrance into the animal's body. The compound was soluble in corn oil, which is comprised of almost 55% linoleic acid and 30% oleic acid (85% unsaturated fatty acids). Poultry fat is 24 and 40% linoleic and oleic acids, respectively (64% unsaturated fatty acids) (Scott et al., 1976). Therefore, solubility in corn oil, a lipid of one type, does not guarantee that the compound would be soluble and become bound to a lipid of another type; particularly when the comparison being made is one of an active metabolic tissue vs. a passive lipid solution. Thus, the fact that DIMP was soluble in a lipid stored in a test tube was in no way a measure of predictability that the compound would have a particular affinity for lipids in the bird's body. As it turned out, the highest ^{14}C values were generally found in organs associated with metabolism and excretion, i.e., kidney and liver.

DIMP as a by-product of nerve gas production would be limited to those environmental sites on which nerve gas was produced or stored. It is slightly soluble in water so ingestion by animal is a possibility should DIMP escape from its burial or dumping grounds and seep into ground water and thence into ponds, streams, rivers, and lakes. Ducks and quail, as wild fowl, could be subject to ingestion of DIMP. Apparently, the Mallard duck has a high tolerance to DIMP when administered per os. Jones (1978) reported that the LD_{50} is 1490 mg per kg of body weight. At a body burden of 1/15 of this amount, i.e., 100 mg per kg body weight used in the dosing experiments, ducks were found to absorb the compound quite readily and have relatively high residues in tissues at amounts ranging from 100 to almost 800 ppm. However, the levels do not persist and almost complete removal of DIMP occurs in 24 to 48 hours. Under such circumstances, ducks flying into watery havens for rest and food and becoming contaminated by ingestion of DIMP would be expected to have only trace amounts of the compound and its metabolites within a couple of days after flying out. The feeding experiment in which the ducks consumed DIMP at 100 ppm, a substantial concentration in the feed, revealed that tissue residue levels of no more than 0.6 ppm would occur if ingestion of DIMP was continuous over a 3 to 5-day period. Within 3 days after departure from such a contaminated area the bird would have less than 40 parts per billion of residue in its tissues. Such wild fowl would not serve as a link in the food chain to pass along DIMP or its metabolites.

The same protocol, with skin as a possible exception, would describe the relationship of the quail consuming DIMP should they be involved within a contaminated area. Quail are less migratory than ducks and reside within certain areas that provide food, water, and cover. If one assumes that a constant supply of DIMP is in their environment and that this supply was at a level of 100 ppm in their diet, then such quail would generally be expected to have about 0.2 ppm of residue in their muscle, and about 0.7 ppm, as an average, for liver, adipose, skin, and kidney. The latter tissues account for approximately 15% of body weight, and muscle for about 40%.

Thus, about 70% of the carcass consumed by the predator of quail would be derived from muscle containing about 0.2 ppm residue. A predator consuming only the organs and muscle of quail, who in turn had been ingesting DIMP at 100 ppm in their diet, would be eating a diet with an average level of residue at 0.35 ppm. Thus, one passage through quail markedly reduces the link in the food chain for DIMP.

Conclusions

Ducks and quail fed diets with radioactive DIMP had ^{14}C residues averaging less than 1 ppm which declined to less than detection limits, averaging 0.04 ppm, in most tissues by the 3rd day after withdrawal. All tissues, but skin, were clear of residues by day 5 off radioactive diets. Skin had 0.05 to 0.1 ppm at that time.

In the dosing experiments, residues were 5.1 to 756 ppm, depending upon tissue and species. Nevertheless, values decreased rapidly with a biological half-life of 12.7 hours. Most tissues were at or below detection limit in 48 hours and would be clear at 65 hours, based on the biological half-life value.

DIMP was not concentrated in adipose tissue of either duck or quail. The rapid biological half-life and lack of binding to fat cells in the carcass indicate that DIMP is not retained for passage along the food chain by predators of these fowl.

Toxicity of DCPD to Mallard Ducks

The DCPD studies were divided into three tests as with DIMP. Test 1 was concerned with the lethal dose for 50 percent of the animals (LD_{50}), test 2 dealt with the lethal chronic level (LC_{50}), and test 3 was a long term chronic study. All three tests utilized Mallard ducks[1], (Anas platyrhynchos). The mallards were procured from two locations: (1) Max McGraw Wildlife Foundation, Dundee, Illinois, 60118; (2) Frost Game Farm, Coloma, Wisconsin, 54930. All tests were conducted in a windowless house at the Michigan State University Poultry Science Research and Teaching Center.

TEST 1 - ACUTE (LD_{50})

Procedure

This test was designed to determine the single oral dose LD_{50} of dicyclopentadiene (DCPD) to the Mallard.

Adult Mallards, approximately one year of age in non-laying condition, were utilized. The birds were held indoors in batteries. The batteries measured 122 cm (l) x 78.7 cm (w) x 35.6 cm (h) and there were ten ducks per battery for 960 cm^2 floor space per bird. The birds were reweighed at the termination of the two weeks to note if any significant weight loss occurred before range finding began.

Preliminary range finding was done to establish the approximate lethal dose and a series of dosages was employed for the test to give mortality ranging from 10 to 90 percent.

Testing

Birds used for testing were maintained on duck breeder developer (Appendix A: Analysis of Feed). This feed was free of antibiotics and medication. Feed and water were provided ad libitum throughout the testing period. Food consumption was determined weekly for all groups. Before oral administration of chemicals, a fasting period of at least 15 hours was utilized.

Twenty birds were used per dose level, ten of each sex, the control groups consisted of ten birds of each sex dosed with water. All birds were weighed before dosing and on days 3, 7, and 14 after dosing. Administration was by drenching per os from a syringe with a length of tubing attached to the needle. The length of tubing used corresponded with the distance from the back of the oral cavity to the esophageal opening of the proventriculus. This insured a uniform location for introduction of the chemicals. The syringe was either 3 cc or 5 cc, the needle was 20 ga, 3.81 cm long, and the tubing measured 1.143 mm ID and 1.575 mm OD. The total volume of

---

[1]Phenotypically indistinguishable from wild Mallards.

chemical had a constant volume to body weight factor per animal. Minimum observation time for each animal was: during the first hour after dosing, four to five hours after dosing, and daily thereafter.

Necropsies were performed on all birds, including controls, at the time of death or at termination of the 14 days of observation. A general gross inspection was performed with special emphasis on the digestive tract, liver, kidneys, heart, and spleen.

Statistical Analysis

The LD_{50} was analyzed by the method of Litchfield and Wilcoxon (1949). Feed consumption was analyzed by ordinary t-test, and approximate t-test. Weight changes were analyzed by one-way analysis of variance with Dunnett t-test.

Results

Determination of acute oral LD_{50} by the method of Litchfield and Wilcoxon (1949) was not possible because no mortality resulted from drug treatment. Therefore, the LD_{50} for DCPD for Mallards was greater than 40000 mg/kg body weight.

Though no deaths occurred with DCPD, responses were noticed. Responses to the 40000 mg/kg dose, which was given 5 cc (~5000 mg) at a time over a maximum of two and one-half hours to prevent drowning, started to appear after approximately 20000 to 30000 mg had been given. Many birds showed no reaction to the chemical other than holding their mouths open during the first part of dosing. Of those that did show a response, only a slight intoxication was noticed and moderate tremors of the head and body in about ten percent of the birds. All the birds appeared to have recovered within two hours after dosing.

During the 14-day post-treatment period, no further signs of intoxication nor significant weight changes were noted for the ducks (Table 70). Necropsies of all birds showed no gross pathological changes attributable to DCPD.

DISCUSSION

Ducks dosed with DCPD up to 40000 mg/kg[1] showed no terminal effects nor any body weight or feed consumption differences over the 14-day post-treatment period (Tables 70-71). This classifies DCPD in the relatively harmless range (see page 29). The LD_{50} (> 4000 mg/kg)

---

[1] For toxicity purposes administration of doses beyond 5000 mg/kg in the acute oral test is not of practical value. Federal Register (1975).

Table 70 Body weight changes of Mallard ducks during 14 day post-treatment observation period following a single per os treatment with DCPD.

Treatment	Treatment level (mg/kg)	n	Mean body weight		Mean change
			Day 0	Day 14	
DCPD	0	20	1113	1151	38_a [1]
DCPD	40000	19	1169	1175	6_a

[1] Means having the same subscript are not significantly different from their control ($P > 0.05$).

Table 71 Feed consumption of Mallard ducks during 14-day post-treatment observation period following a single per os treatment with DCPD.

Treatment	Treatment level (mg/kg)	n	Day 0-7[1] g/b/d	Day 8-14[1] g/b/d
DCPD	0	20	57.28 ± 0.531	49.45 ± 0.539
DCPD	40000	19	44.28[2] ± 0.545	55.40[2] ± 0.553

[1]Data reported as treatment mean ± standard error.

[2] Significantly different from control ($P > 0.0005$).

is more than 114 times the average mammalian LD_{50} of 350 mg/kg and more than 40 times the Bobwhite LD_{50} of 1010 mg/kg presented in this report. Therefore, the Mallard lies outside the general rule of response within a 10-fold range. It may be that DCPD is not absorbed from the gastrointestinal tract in any significant amounts in ducks.

A list of compounds with LD_{50}'s from Tucker and Crabtree (1970) is presented in Table 4 along with LD_{50}'s of DCPD as a comparison of relative toxic levels. Toxicity index, as calculated from Sun (1950), equals the LD_{50} of the Standard/LD_{50} of the sample x 100. For DCPD, the index is less than 0.95. As the route of administration is one of the most influential factors in modifying the LD_{50}, this index gives a more constant number for comparison between different routes of administration. Though DCPD did not kill ducks when administered in a single dose, this can be misleading. Coburn and Treichler (1946) could not kill ducks or starlings with a single dose of DDT, nor were robins killed by DDT in an LD_{50} study by Hickey and Hunt (1960). Yet, DDT has very toxic cumulative properties. Also, Dougherty (1962) could not kill Mallard ducklings with Korlan if they were allowed to regurgitate.

TEST 2 - SUBACUTE (LC_{50})

Procedure

This subacute test was designed to determine the maximum repeated dosage tolerable to Mallard ducklings on DCPD-treated diets. A random selection of healthy twelve-day-old ducklings was employed for two reasons: (1) to avoid any possible interference of chemical intake by the yolk sac absorption and (2) to exclude any late hatching mortality. Sex of the bird was not taken into account, because determination of sex was not practical for birds of this age. The ducklings were held indoors in a Petersime Brood unit[2] from one day of age through the end of the test.

A range finding pilot test was performed to determine the effect on feed consumption and body weight. A series of dosages was employed in the test to determine the point of zero feed consumption rather than 50 percent mortality, since no deaths occurred during range finding.

Testing

The ducklings were maintained on duck starter diet (Appendix A: Analysis of Feed). This feed was free of antibiotics and medication. Feed and water were provided *ad libitum* throughout the testing period. The test ran a total of eight days; the treated diets were fed for the first five days and untreated feed was provided for the

---

[2] Petersime Incubator Co., Gettysburgh, OH 45328

last three days. The three days post-treatment period was used to avoid bias due to overestimating the dose by not taking into account mortality that would not have occurred because the compounds did not have time to act. Treated feeds were prepared by adding a chemical: corn oil solution to the duck starter (Appendix B: Diet Preparation). Because DCPD appeared to be relatively harmless (LD_{50} greater than 15000 mg/kg), the chemical-corn oil solution was greater than two percent. For DCPD, ten dietary treatments were used: 0, 10000, 20000, 30000, 40000, 50000, 60000, 70000, 80000, and 90000 ppm. Ten ducklings of undetermined sex were placed on each dietary treatment. Because all DCPD-fed groups of ducklings in the initial test showed decreased feed consumption as compared to the control, the experiment was repeated using lower DCPD levels for a longer period of time. Young adult, male Mallards 23 weeks old ±1 week were utilized. Diets used contained the following levels of DCPD: 0, 10, 100, 1000, 5000, and 10000 ppm (Appendix B: Diet Preparation). The birds were fed the treated diet for 32 days at the end of which necropsies were performed on all animals.

All signs of intoxication and abnormal behavior were noted throughout the eight days and all surviving animals were necropsied at the end of the test.

Estimates of average feed consumption with observation on excess spillage were made for determination of maximum repellency (estimated zero feed consumption).

Statistical Analysis

Slopes of feed consumption and body weight changes and predicted zero feed consumption were determined by regression analysis.

Results

Results of the five day range finding trial were:

Treatment	Level in diet (ppm)	Change in body wt. g/b/d	Feed consumed g/b/d	Percent mortality
DCPD	20000	21.1	39.32	0
DCPD	30000	17.4	31.66	0

Since DCPD did not cause any mortality during the acute test nor during range finding, seven of the ten levels were set above the maximum two percent levels recommended in the Federal Register (1975). This was done to establish a zero feed intake level if mortality did not reach 50 percent at any level.

The feed consumption of the ducklings on diets containing DCPD (Figure 21) was decreased in all treated groups as compared to the control group. This decrease ranged from 28.7 percent for birds

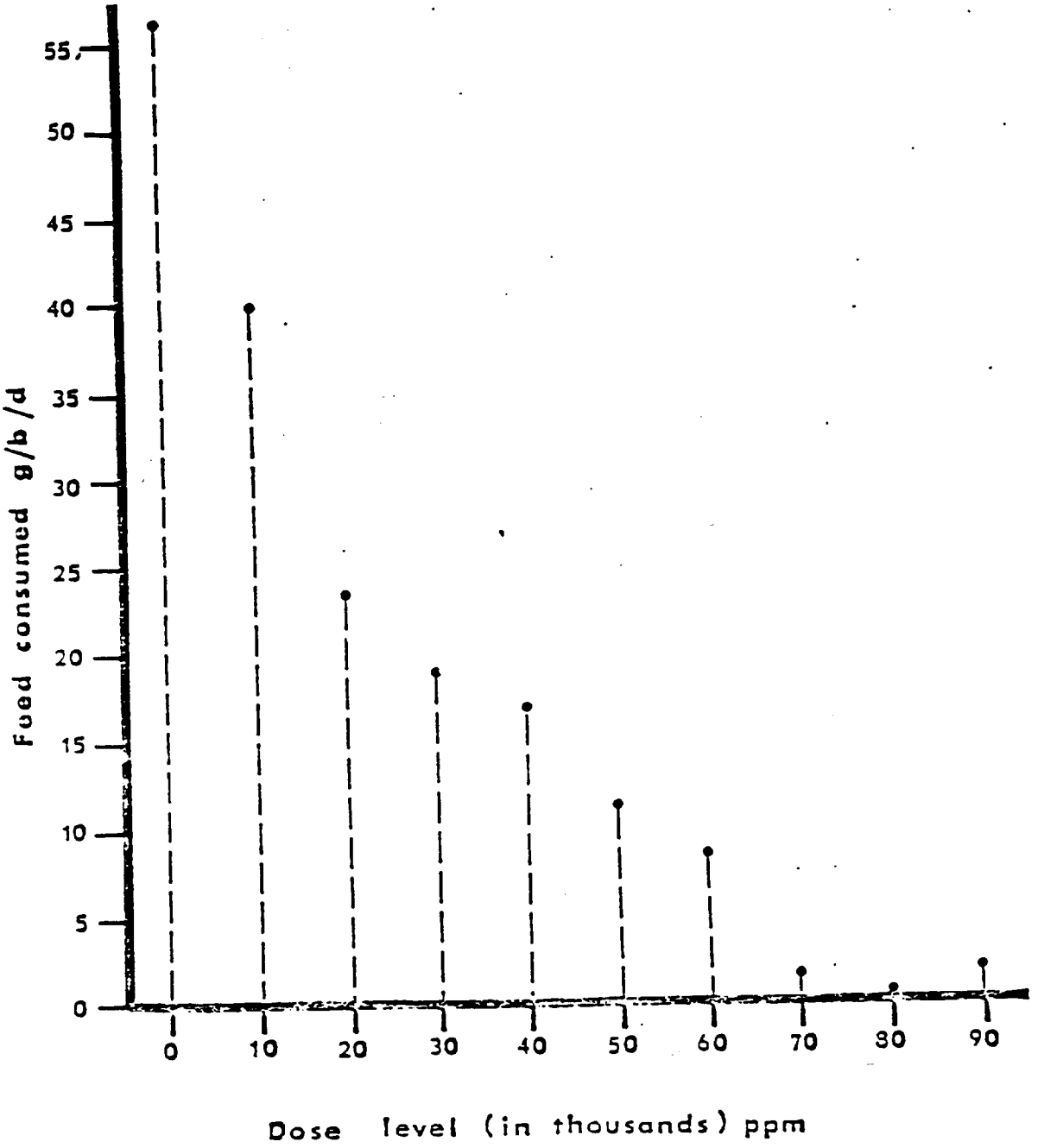

Figure 21. Effect of feeding DCPD at various levels in the feed for 5 days on feed consumption of 12-day-old Mallard ducklings.

receiving the 10000 ppm diet to 98.7 percent for those receiving the 80000 ppm diet. The feed consumption of those ducklings receiving the three highest levels of DCPD (70000, 80000, and 90000 ppm) was nearly zero (mean of 1.41 g/b/d for the three groups). The 10000 and 20000 ppm groups had the steepest rate of decline in feed consumption (Figure 22) with a slope of -0.0017 (or -1.657[1]) and a correlation between feed consumption and level of DCPD in the diet of -0.99987. The higher treatment groups, 30000 to 70000[2], showed a smaller rate of decline with a slope of -0.4121; the predicted zero feed consumption was calculated off this line to be 77290 ppm DCPD in the diet. Body weight changes (Figure 23) showed that all treatment groups, with the exception of the birds on the 10000 ppm diet, lost from 1.64 to 19.08 g/b/d with an average loss of 6.74 g/b/d. Total intake of the chemical ranged from 340 to 3312 mg/kg/day (Table 72) with the least amount of intake in the three highest groups (70000, 80000, and 90000 ppm) since they had refused to consume the feed. Mortality ranged from 0 to 30 percent (mean of 8 percent) and showed no trends (Table 72). The highest mortality occurred in the 60000 ppm group which consumed over 3000 mg/kg/day of chemical, but the 40000 ppm group which also consumed over 3000 mg/kg/day of DCPD had no mortality. Correlation between mortality and mg DCPD/kg/day ingested was only 0.441. During the three-day post-treatment period groups previously on diets containing 30000 ppm, or more, DCPD had increased feed consumption over the control from 24.4 percent at 70000 ppm to 36.7 percent at 50000 ppm (Figure 24) with a mean increase of 32.2 percent (8.22 g/b/d). This increase is not present in the 10000 and 20000 ppm groups during the post-treatment as they were an average 15.2 percent (3.87 g/b/d) less than control birds. Body weight gains during post-treatment (Table 73) in the lower groups, 10000 to 40000 ppm, were 2.5 to 7.7 g/b/d with a mean gain of 5.81 g/b/d which was 2.45 g/b/d greater than the control; while the higher groups, 50000 to 90000 ppm, gained 21.4 to 29.7 g/b/d with a mean gain of 25.6 g/b/d which was 22.2 g/b/d more than the control birds.

In the DCPD treated repeat group of Mallards (Table 74) feed consumption was not affected by any level of the drug, but body weight was lost in increasing amounts by birds receiving the three highest levels; for 1000 ppm a decrease of 30.8 g/b from the control, the 5000 ppm was 83.9 g/b lower, and the 10000 ppm group was decreased by 183.6 g/b. Ingestion of DCPD ranged from 0.505 to 736.24 mg/kg/day and no mortality occurred during the 32 day period. There was a correlation of -0.992 between level of drug in the diet and body weight change.

Necropsies showed no gross pathological changes in treated groups from controls.

---

[1] Equals -1.657 when calculated with dose divided by 1000. All subsequent slopes will be given in this manner.

[2] The 70000 to 90000 ppm groups were averaged and used as one point for regression analysis since none of the groups apparently ate any feed, but rather "tasted" it daily, thus giving a small calculated feed consumption.

Figure 22. Regression equations of the data shown in Figure 21. In the regression equations x = ppm of DCPD in the feed and y = feed consumption in g/b/d.

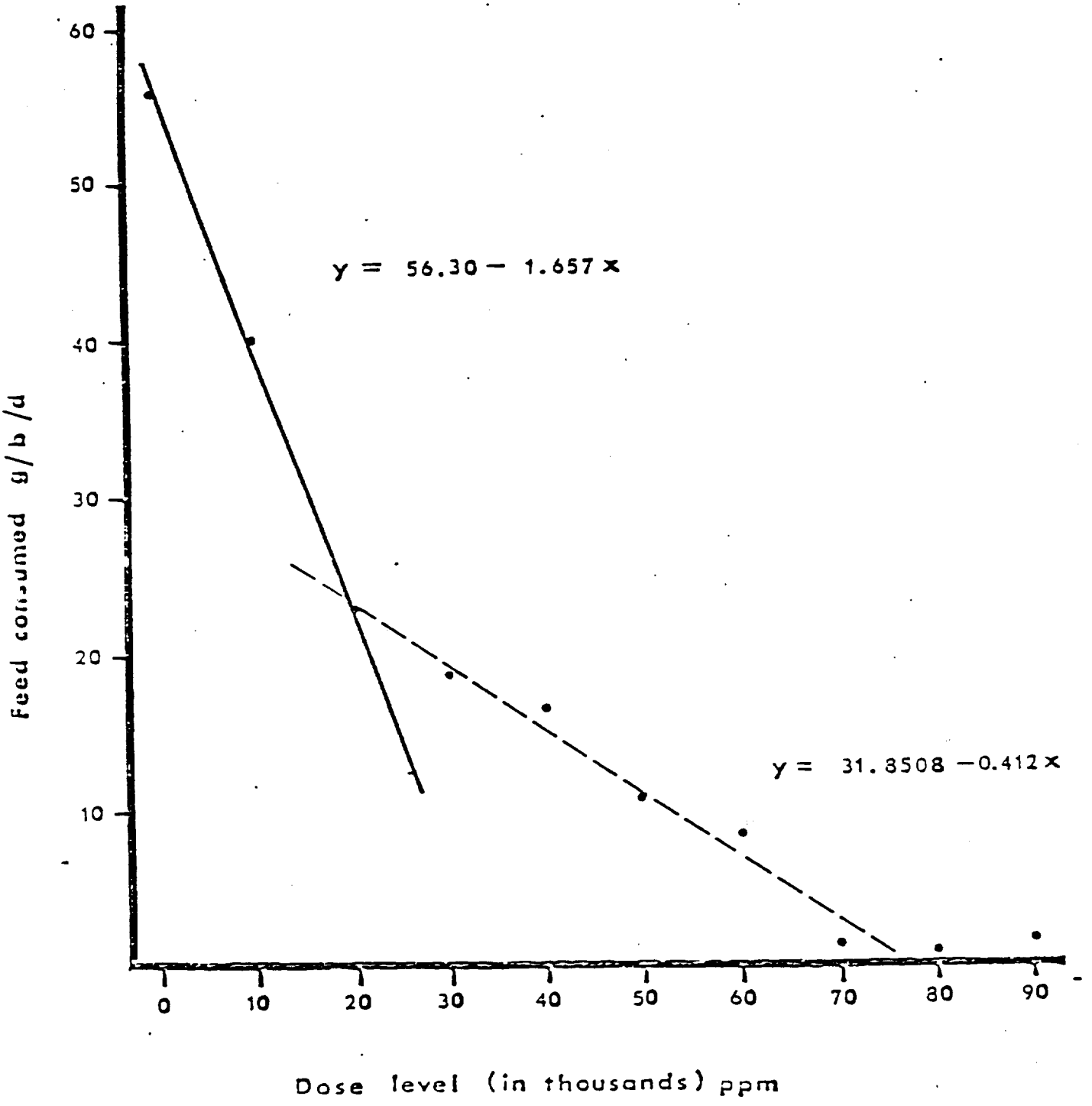

Figure 23. Effect of feeding DCPD at various levels in the feed for 5 days on body weight change of 12-day-old Mallard ducklings.

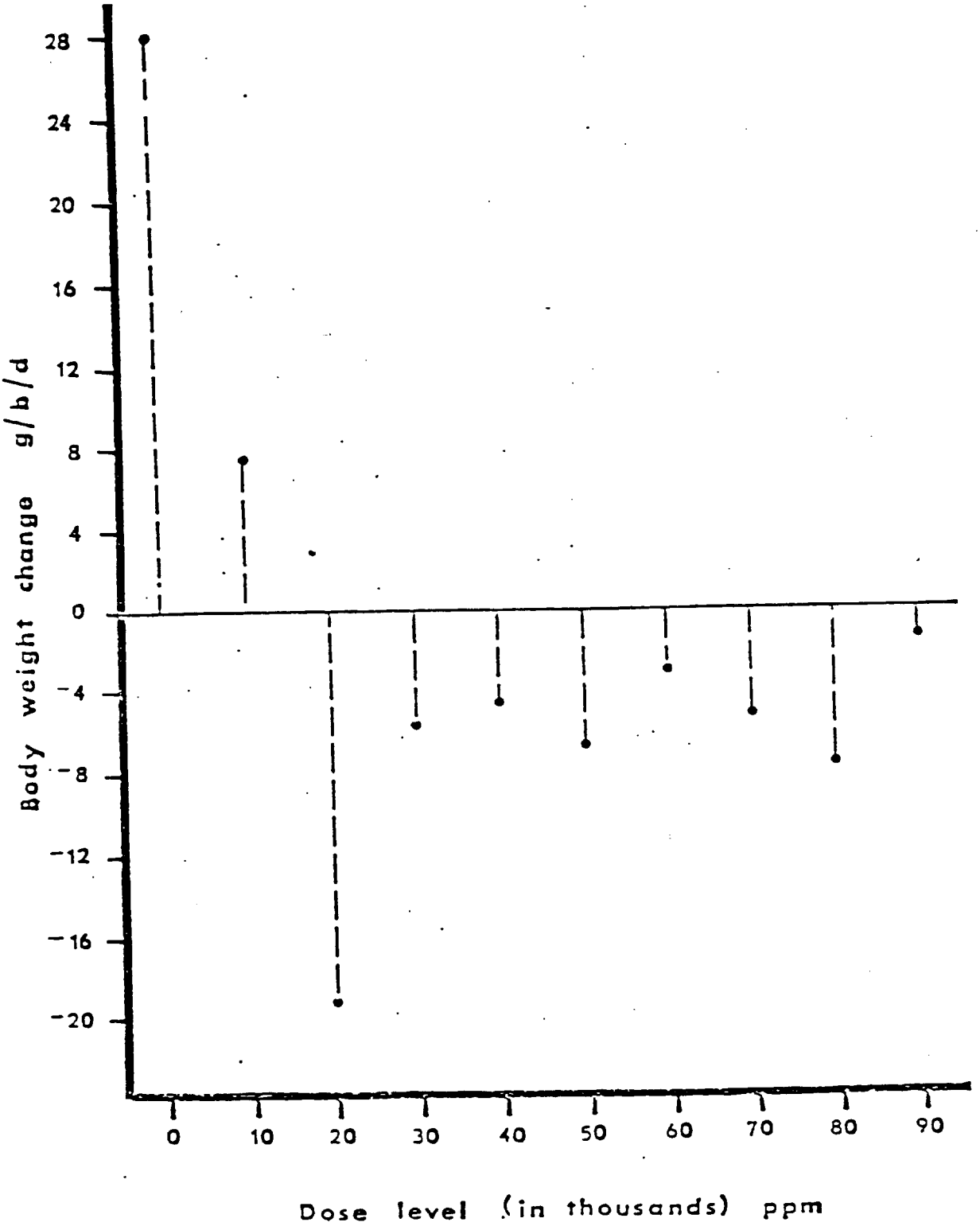

Table 72 . Calculated DCPD intake over 5 days and mortality over 8 days for 12-day-old Mallard ducklings on LC_{50} trial

Dose (ppm)	DCPD consumed/day (mg)	Mean body wt. (g)	mg DCPD/ kg/day	Mortality %
0	0	277.3	0	0
10,000	400.4	246.9	1621.7	0
20,000	460.0	240.7	1911.1	20
30,000	564.6	222.7	2535.2	10
40,000	674.4	203.2	3318.9	0
50,000	555.0	203.6	2725.9	10
60,000	519.0	170.7	3040.4	30
70,000	120.4	171.3	702.9	0
80,000	56.8	162.9	348.7	10
90,000	162.0	172.2	940.8	0

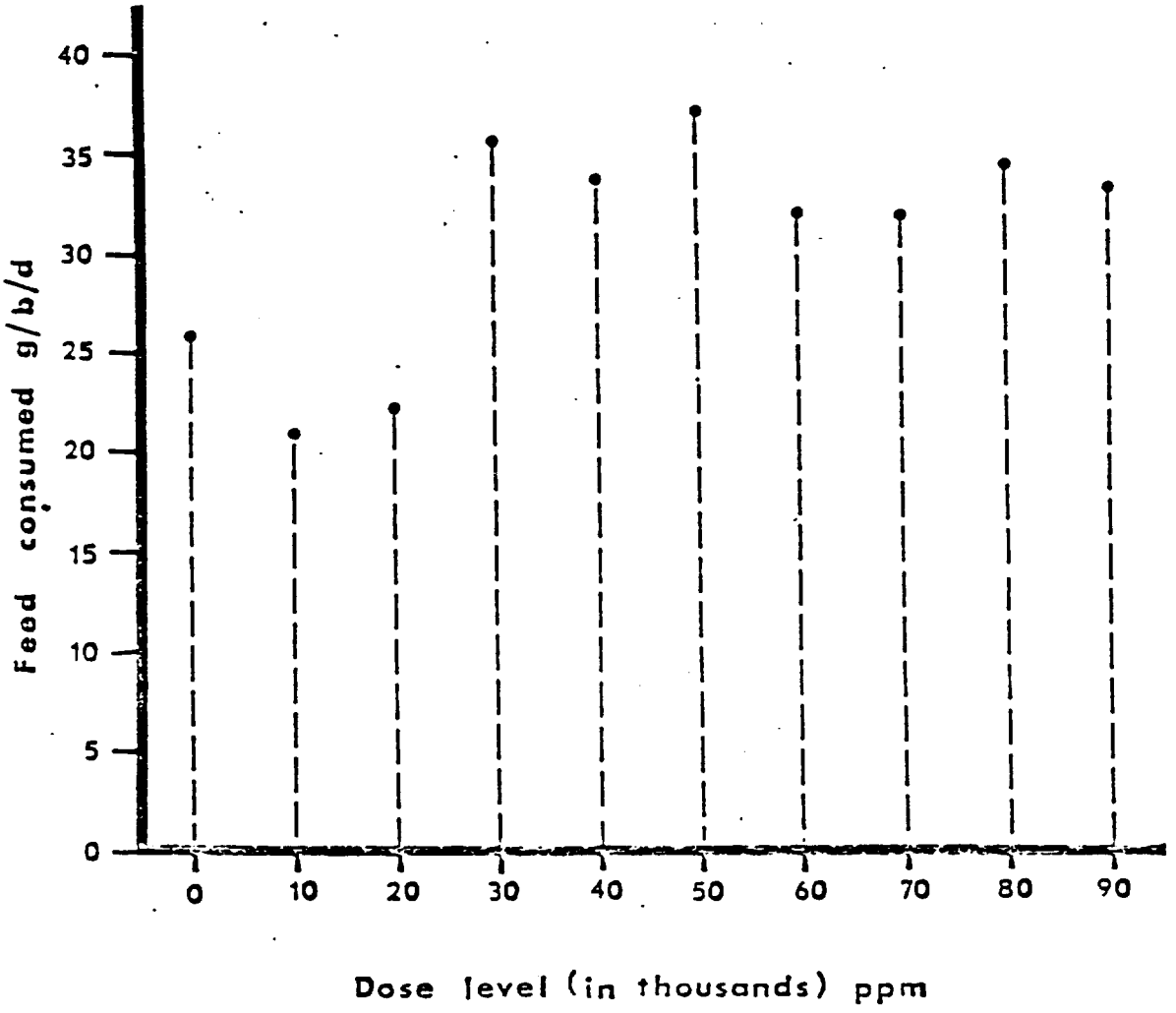

Figure 24. Feed consumption of 17-day-old Mallard ducklings fed non-treated diet during 3-day post-treatment after withdrawal of DCPD-treated diet.

Table 73 . Body weight gain of 17-day-old Mallard ducklings during 3-day post-treatment on non-treated feed after withdrawal of DCPD-treated feed.

DCPD level in the diet (ppm)	Weight gain g/b/d	Feed consumed/ weight gain
0	3.36	7.59
10,000	2.46	8.51
20,000	6.50	3.43
30,000	6.55	5.44
40,000	7.73	4.29
50,000	23.66	1.55
60,000	29.20	1.09
70,000	21.40	1.48
80,000	29.70	1.15
90,000	24.00	1.38

Table 74 . Feed consumption, body weight change, and amount of chemical ingested by 23-week-old male Mallards, fed diets treated with DCPD, at various levels, for 32 days

DCPD level in diet (ppm)	Mean weight gain (loss)/bird (gms)	Feed consumed[1] g/b/d	Days	DCPD consumed/day (mg)	Mg DCPD/ kg/day
0	17.4	67.11 ± 3.52$_a$[2]	1-14	0.0	0.0
0			15-32	0.0	0.0
10	9.0	66.31 ± 3.52$_a$	1-14	0.568	0.505
10			15-32	0.710	0.644
100	27.2	66.22 ± 3.52$_a$	1-14	5.30	4.75
100			15-32	7.45	6.39
1000	(13.4)	66.33 ± 3.52$_a$	1-14	56.9	46.00
1000			15-32	71.2	59.88
5000	(66.5)	66.02 ± 3.52$_a$	1-14	284.3	249.39
5000			15-32	351.0	319.67
10000	(166.2)	66.34 ± 3.52$_a$	1-14	569.0	543.46
10000			15-32	709.0	736.24

[1]Data reported as treatment mean ± standard error.

[2]Means with the same subscript are not significantly lower than the control group ($P > 0.05$).

Discussion

The lethality of a chemical mixed in the diet can differ markedly from that of the pure chemical administered as a single oral dose (Stickel et al., 1965). There was no lethality difference for DCPD.

A comparison of LC_{50} values taken from Heath et al[3]., (1972) is listed in Table 7. There are a number of compounds with no LC_{50} determinations, mostly in non-insecticides, as there was little or no mortality. Of 12 compounds given in order of relative toxicity (see Table 8), DCPD placed low on the list; thus, it is less toxic in comparison to most other compounds used in commerce.

The decreased food consumption at levels above 20000 ppm for DCPD-treated ducklings was probably due to a refusal to eat the very high concentrations of chemical (odor was very strong) and not due to an altered appetite. When placed on clean feed, they consumed more than the control group (Figures 21 and 24). If appetite was affected by DCPD then its effect must have been of very short duration, as there was no intake effect during post-treatment. All ducklings that consumed feed with more than 20000 ppm DCPD when put on regular feed increased their intake above the control. This increase in consumption was apparently an attempt to compensate for their lack of intake during the preceding five days of subjection to a treated diet (Figure 24).

The DCPD-treated repeat group of Mallards showed no effect until the level of DCPD in the diet reached 1000 ppm (Table 74) at which level body weight was lost; though feed consumption was not affected by any level. This finding may have been because these ducks were older and were not affected by the chemical via repulsion or decreased appetite, but by some unknown mechanism causing decreased uptake of nutrients. A decreased uptake could be at either the intestine, by slowing absorption of nutrients, or the liver, where enzyme activity may be decreased; thus not allowing for enough endogenous constituents to be available for both conjugation and normal growth (Dinman, 1974).

Body weights for ducklings treated with DCPD were not affected in the same manner as was feed consumption. All groups fed over 10000 ppm lost weight with the 20000 ppm group losing the most even though they ate more than any higher concentration group (Figure 73). If the chemical had affected uptake of nutrients, then the 30000 and 60000 ppm groups should have lost as much, if not more, weight than the 20000 ppm group as they took in more mg/kg/day of the chemical (Table 72). During the post-treatment period, all groups previously on DCPD, except 10000 ppm, were more efficient in their feed utilization (Table 73) than was the control as the feed

---

[3] Except for DDT on 5-7 day old Mallard ducklings from Heath and Stickel (1965) and Mallards treated with DIMP or DCPD from this study.

consumption/body weight gain ratio was less than that of the control. All groups above 40000 ppm had feed efficiencies of less than 1.60 or at least 4.75 times better than the control.

In the repeat group of DCPD-treated Mallards, the loss of weight in the three highest levels , 1000, 5000, and 10000 ppm, was proportional to the ppm in the diet. The 5000 ppm group lost 4.96 times as much weight as the 1000 ppm group and the 10000 ppm group lost 12.4 times as much weight as the 1000 ppm group.

TEST 3 - CHRONIC

Procedure

This test was designed to determine the toxicological effects on adult Mallards and their progeny from continuous exposure to DCPD over a reproductive cycle.

Four test groups of randomly selected ducks were used. One group served as a control and three groups as treatment birds. Each group consisted of a pen of two males and five females and was replicated three times. All groups were randomly assigned to pens. The size of each pen was 1.47 m x 1.55 m x 0.7 m high with no top. Wing feathers were clipped to prevent the birds from escaping.

Testing

Diets were prepared by adding a chemical-corn oil solution to the pelleted feed (Appendix B: Diet Preparation). The control diet consisted of corn oil at two parts mixed to 98 parts of pelleted feed. Water and prepared diets were provided ad libitum throughout the entire 22 weeks. The animals were on the treated feed a minimum of ten weeks before commencement of egg production and a minimum of ten weeks after 50 percent production level was attained. Duck breeder developer feed was fed for the first six weeks and breeder layer feed was fed for the remainder of the trial. Food consumption was measured at biweekly intervals during the entire test.

The room was kept at approximately 7°C and six hours of light/day before egg production (December 28 to March 3) and raised to approximately 12.8°C and 19 hours of light/day to induce egg production. Temperatures ranged from 8.3°C to 32.3°C for the rest of the study (March 4 to June 2). The higher room temperatures generally occurred toward the end of the test.

Body weights were taken at weeks 0, 2, 4, 6, 8, and at termination of treatment. During egg laying no weights were taken because of the adverse effects that handling may have had on egg production.

Mortality was recorded along with gross pathology of the animals. Morbidity and clinical signs were observed throughout the study.

Any animals that died were necropsied, a gross examination performed, and the following organs weighed: liver, spleen, kidneys, pancreas, proventriculus, gizzard, gonad(s), heart, and brain.

Egg Collection, Storage, and Incubation

Percent egg production was based on hen-day production, where each day's collection is divided by the number of hens alive and multiplied by 100 to get a percentage. Eggs were collected and marked daily from each pen and stored at 12.8 to 15.6°C. Eggs were set once a week in a Jamesway, single stage, 252 incubator[3]. The eggs were incubated for 23 days at an average temperature of 37.5°C, with a range from 36.9°C to 38.1°C, and at an average relative humidity of 56 percent, with a range from 52 to 65 percent. After the first 23 days of incubation, the eggs were transferred to a hatching unit at an average temperature of 37.2°C, with a range from 36.8°C to 38.1°C and a relative humidity of 65 to 70 percent. All eggs were candled on day 0 for shell cracks and on day 14 of incubation to measure fertility and early deaths of embryos. All eggs that did not hatch were checked for abnormalities and placed in one of the following categories: dead in shell, live in shell, pipped live, or pipped dead.

At hatching all ducklings were wing banded and housed in a Petersime battery brooder and observed for two weeks while on duck-starter. Mortality of all ducklings was recorded for the 14-day period and percent livability calculated.

At biweekly intervals all eggs from one day's collection were measured for eggshell thickness. Eggs to be measured were cracked open at the girth, contents washed out, and shells air dried for at least 48 hours before thickness was determined. Measurements were taken of the dried shell plus the shell membranes at four points around the girth using a micrometer[4] calibrated to 0.01 mm units.

Histopathology

At the termination of the test all surviving animals were killed by cervical dislocation, a gross examination of the carcasses performed and the organs (liver, spleen, kidney, pancreas, proventriculus, gizzard, heart, and brain) excised and weighed. A sample of these organs plus lungs, adrenals, duodenum, and sciatic nerve were then placed in ten percent neutral buffered formaldehyde (Luna, - 1968) and prepared for histopathologic examination according to routine procedures, as described in Appendix C.

---

[3] James Manufacturing Company, Inc. (a subsidiary of Butler Manufacturing Co.), Fort Atkinson, WI 53538

[4] Federal Products Corp. (a subsidiary of Esterline Corp.), 1144 Eddy Street Providence, RI 02901

Hematological Preparation

Hemoglobin concentration, packed red cell volume (hematocrit value), and differential counts were determined for all birds at the termination of the experiment (see Appendix D, E, and F).

Statistical Analysis

Treatment groups were compared to their respective control by analysis of variance. Sample units were the individual pens within each experimental group except for body weights, organ weights, and hematology where sample units were the individual animals. Egg production and feed consumption were analyzed by split-plot design (Gill, 1978).

Results

The reproduction period was chosen as it offers a unique set of physiological and behavioral conditions in both parents and progeny. The endocrine changes in the parents, and embryo and prenatal developments in the young may accentuate any toxicological effects from the addition of a substance to the diet. Most notable effects are embryo mortality and teratogenicity, the induction of fetal malformations.

The purpose of the reproductive test was to establish an exposure level that may be absorbed over a long period without producing any toxicological effects characteristic for the same chemical when given in larger amounts; since a chemical may be innocuous in terms of acute mortality but still impair reproduction. Thus, if a compound significantly decreased spermatogenesis in the drake or had an adverse effect on the ovaries of the hen, then a decrease in fertility would result or possibly a decrease in numbers of eggs laid, such as reabsorption of developing follicles. Another objective was the determination of the long-term effects, if any, such as degenerative or carcinogenic changes, and/or unsuspected behavioral or physiological reaction not previously observed.

For the chronic study, including reproduction, animals were given the test substance in the feed for a period (minimum of 10 weeks) prior to onset of egg laying, and drug administration was continued throughout the reproductive cycle. Levels of chemical employed in the chronic test were derived from the subacute test[3]. Thus, DCPD, which adversely affected body weight gains at levels of 1000 ppm and above, was set at 320 ppm and below.

---

[3] Data from the repeat group of DCPD-treated Mallards in test 2 were used more in determining the levels of DCPD to be used in the chronic test than the first trial group.

Chemical intake is stated as ppm and not as mg/kg/day as in test 2. Expressing dose in mg/kg/day can be misleading when animals are exposed over a long time. Animals that die early, and have consumed less in terms of milligrams than surviving birds, point to the erroneous conclusion that lower dosages of a drug are more toxic than higher dosages. Furthermore, an accurate measurement of mg/kg/day is impossible during the egg laying period as birds would have to be weighed periodically. This handling might stress them sufficiently to cause cessation of egg laying or even cause mortality. Also, excretion of chemical through the urine and feces would need to be measured and chemical content determined to measure excretion of chemical per day, thus giving level of chemical in the body per day.

Feed consumption is plotted in Figure 25 for the ducks treated with DCPD. Each point plotted is the mean of three cages of seven ducks per cage. There was no significant difference in any DCPD-treated group as compared to its control.

Mean body weight changes are reported in Table 75. There was no significant difference in body weight change of any DCPD-treated group as compared with its control group.

Body weight changes from before start of egg laying to end (or near end) of the egg production period are listed in Table 76. All treated groups gained weight with no significant difference between treated groups and the control.

Egg production for DCPD-treated ducks is plotted in Figure 26. Each point plotted is the mean of three cages of five hens per cage. Percent production was based on hen-day production. There was no significant difference between the treated groups' overall egg production as compared to the control.

Eggshell thickness for DCPD-treated Mallards is listed in Table 77. No significant difference was found between treated groups and the control. All eggs used for eggshell thickness measurements were not included in any calculated percentages other than production.

Incubation parameters for the ducks on DCPD-treated feed are listed in Table 78. There was no significant difference between any treated group and the control in any parameter. The values for percent fertile eggs are based on the number of settable eggs. Percent hatchability, early dead, dead in shell, live in shell, pipped live, and pipped dead are based on the total number of fertile eggs. Livability of all ducklings for the 14-day period after hatching is listed in Table 79. There was no significant difference between any treated group of parents' ducklings and the control parents' ducklings.

Histopathologic examination of the tissues taken from the treated groups of Mallards revealed no differences from the controls.

Table 74 . Feed consumption, body weight change, and amount of chemical ingested by 23-week-old male Mallards, fed diets treated with DCPD, at various levels, for 32 days

DCPD level in diet (ppm)	Mean weight gain (loss)/bird (gms)	Feed consumed[1] g/b/d	Days	DCPD consumed/day (mg)	Mg DCPD/ kg/day
0	17.4	67.11 ± 3.52$_a$[2]	1-14	0.0	0.0
0			15-32	0.0	0.0
10	9.0	66.31 ± 3.52$_a$	1-14	0.568	0.505
10			15-32	0.710	0.644
100	27.2	66.22 ± 3.52$_a$	1-14	5.30	4.75
100			15-32	7.45	6.39
1000	(13.4)	66.33 ± 3.52$_a$	1-14	56.9	46.00
1000			15-32	71.2	59.88
5000	(66.5)	66.02 ± 3.52$_a$	1-14	284.3	249.39
5000			15-32	351.0	319.67
10000	(166.2)	66.34 ± 3.52$_a$	1-14	569.0	543.46
10000			15-32	709.0	736.24

[1]Data reported as treatment mean ± standard error.

[2]Means with the same subscript are not significantly lower than the control group ($P > 0.05$).

Table 75. Effect of feeding DCPD at various levels in the diet for eight weeks before commencement of egg production on body weight changes of adult Mallards.

Treatment	Level in the diet (ppm)	n	Mean body weight change					
			Weeks 1-4		Weeks 5-8		Combined	
			gms	As a % of body wt.[1]	gms	As a % of body wt.[1]	gms	As a % of body wt.[1]
DCPD	0	21	- 8.69	-0.70	-5.02	-0.41	-13.71_a[2]	-1.11
DCPD	32	21	-19.86	-1.67	-5.55	-0.49	-25.41_a	-2.16
DCPD	100	21	- 8.48	-0.44	-1.62	0.09	-10.10_a	-0.35
DCPD	320	21	-15.62	-1.31	-2.16	0.24	-13.46_a	-1.07

[1]Average percentage change by individual.

[2]Numbers with the same subscript are not significantly different from their respective control ($P > 0.05$).

Table 76. Effect of feeding DCPD at various levels in the diet before egg production starts and after egg production commences on body weight change of adult Mallards during their first reproductive cycle.

Treatment	Level in the diet (ppm)	Mean body weight (gms)		Change	
		Before production	End of production	%	BW/gms
DCPD	0	1175.7	1295.7	10.21	120.0_a[1]
DCPD	32	1185.3	1252.2	5.66	67.1_a
DCPD	100	1241.6	1319.4	6.27	77.8_a
DCPD	320	1200.3	1306.9	8.88	106.6_a

[1]Numbers with the same subscript are not significantly lower than their respective control group ($P > 0.05$).

Figure 26. Effect of feeding DCPD at various levels in the diet for 22 weeks on egg production of adult Mallard hens in their first reproductive cycle. Each point represents the mean of three cages of five females each. Percents calculated from hen-day production.

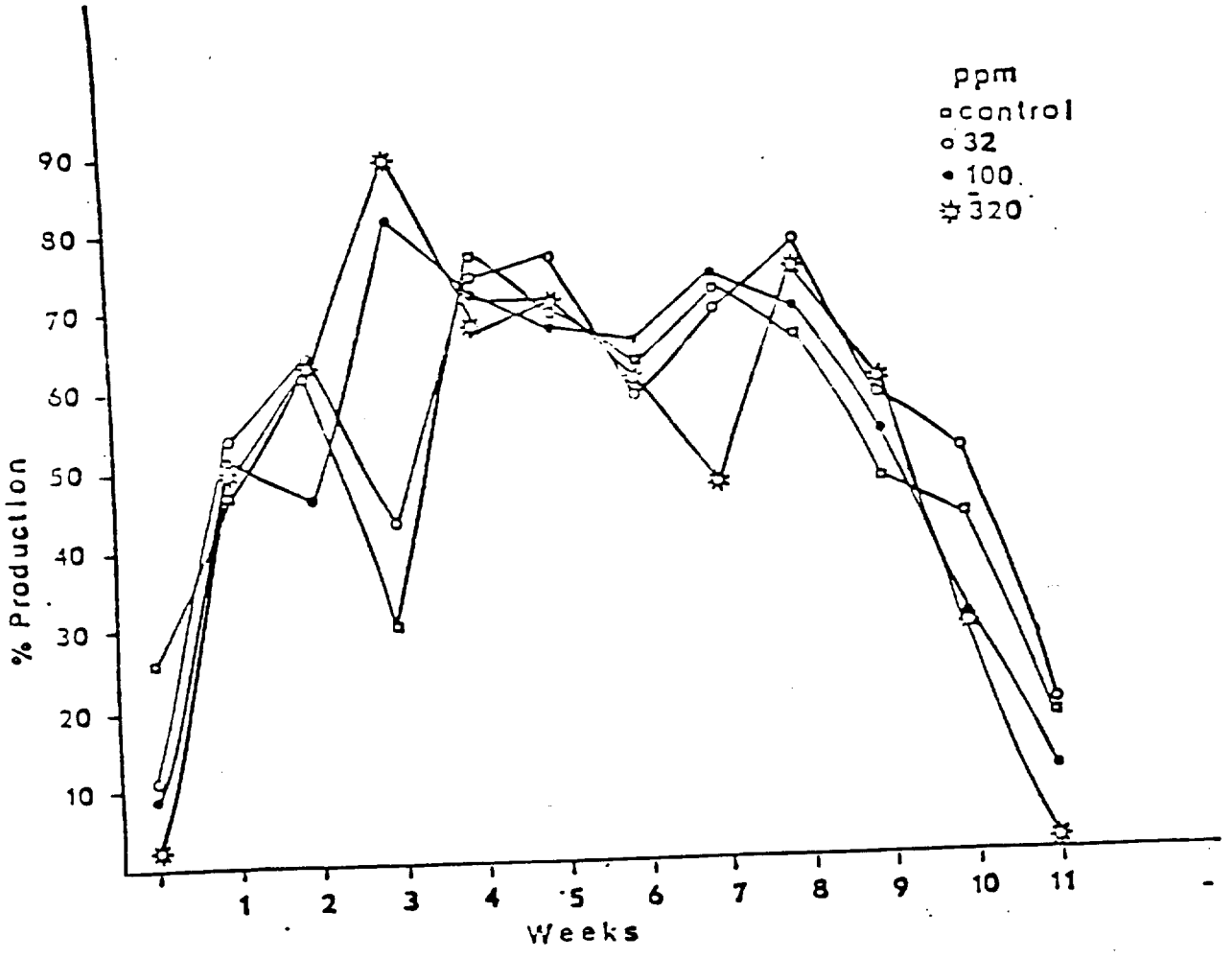

Table 77. Effect of feeding DCPD at various levels in the feed for 22 weeks on eggshell thickness values of adult Mallard eggs from females during their first reproductive cycle.

Treatment	Level in the diet (ppm)	Cage	N	Mean thickness[1] ($mm \times 10^{-2}$)	Combined N	Combined Mean
DCPD	0	6	18	40.7 ± .775		
	0	10	19	41.8 ± .552	53	$40.90 \pm .374_a$[2]
	0	18	16	40.0 ± .734		
	32	4	16	40.8 ± .655		
	32	11	23	40.0 ± .719	55	$39.99 \pm .367_a$
	32	20	16	29.2 ± .815		
	100	2	15	39.7 ± .595		
	100	15	19	39.7 ± .525	57	$39.29 \pm .364_a$
	100	24	23	38.6 ± .426		
	320	3	22	41.1 ± .600		
	320	7	18	40.6 ± .563	56	$41.10 \pm .361_a$
	320	17	16	41.6 ± .536		

[1] Data given as group mean ± standard error.

[2] Numbers with the same subscript are not significantly different from their respective control ($P > 0.05$).

Table 78. Effect of feeding DCPD at various levels in the diet for 22 weeks on incubation parameters of Mallard duck eggs laid in March, April, and May, 1977

Parameter (%)	Level in diet (ppm)	March	April	May	Combined
Cracked	0	3.90	5.78	3.29	4.51_a[1]
	32	5.39	4.69	2.06	4.29_a
	100	3.49	7.92	7.24	6.23_a
	320	2.14	4.21	2.69	3.18_a
Fertile	0	80.40	92.06	63.27	81.64_b[1]
	32	93.50	82.53	67.83	83.69_b
	100	63.86	66.42	65.25	65.38_b
	320	89.78	91.19	95.51	89.29_b
Hatched	0	61.35	62.35	59.14	61.46_c[1]
	32	76.52	67.84	63.92	69.16_c
	100	87.74	68.18	65.22	72.99_c
	320	62.60	51.68	52.56	54.54_c
Early dead	0	3.36	7.45	5.38	6.00_d[1]
	32	3.48	5.49	4.12	4.71_d
	100	6.60	3.41	5.44	4.81_d
	320	5.69	7.56	4.49	6.19_d
Dead in shell	0	15.13	25.10	34.41	24.41_e[1]
	32	9.57	21.57	26.80	19.70_e
	100	4.72	20.46	28.26	17.91_e
	320	14.63	33.19	33.33	28.82_e
Live in shell	0	3.36	0.39	0.00	1.07_f[1]
	32	0.00	0.00	0.00	0.00_f
	100	0.94	0.00	0.00	0.27_f
	320	0.81	0.84	0.00	0.58_f
Pipped live	0	11.76	3.14	0.00	4.71_g[1]
	32	9.57	4.71	3.09	5.57_g
	100	0.00	7.39	0.00	3.48_g
	320	11.38	5.88	7.69	7.74_g
Pipped dead	0	5.04	1.57	1.08	2.36_h[1]
	32	0.87	0.39	2.06	0.86_h
	100	0.00	0.57	1.09	0.53_h
	320	2.44	0.84	1.92	1.55_h

[1]Means with the same subscript are not significantly different from their respective control ($P > 0.05$).

Table 79. Effect of feeding DCPD at various levels in the diet over the first reproductive cycle on the mean 14-day livability of progeny over 16 hatch periods, one hatch/week

Treatment	Level in parents' diet (ppm)	Percent of hatched ducklings alive at end of 14 days	No. diet/ no. hatched
DCPD	0	98.61_a[1]	4/287
	32	98.76_a	4/323
	100	99.27_a	2/273
	320	99.29_a	2/282
Total		98.97	12/1165

[1]Means with the same subscript are not significantly different from their respective control ($P > 0.05$).

Hemoglobin (Hb) and hematocrit values for DCPD-treated groups of Mallards are listed in Tables 80 and 81. There was no significant difference by sex nor by level of chemical in the diet as compared to the control group. Mean corpuscular hemoglobin concentration (MCHC) was determined by the formula: MCHC = (Hb x 100)/Hct, where Hb equals hemoglobin gm/dl and Hct equals packed cell volume. MCHC is listed in Table 82 for DCPD-treated ducks. Ranges for DCPD-treated Mallards were 26.82 to 30.92 percent for 0 ppm, 25.81 to 31.90 percent for 32 ppm, 26.03 to 30.70 percent for 100 ppm, and 25.00 to 30.23 percent for 320 ppm. There was no significant difference in MCHC between sexes, nor between treatment levels as compared to the control group. Leukocyte counts of the Mallards treated with DCPD are listed in Table 83. There was no significant difference between any treated group and its control for any type of leukocyte.

There was no significant difference in any organ weight on any treatment level as compared to the organ weight of the controls. Organ weights for DCPD-treated animals are listed in Tables 84 and 85. The ducks were divided into male, females with developing follicles, and females without developing follicles. There were very few males in a reproductive state at the time of termination and, thus, they were not divided into reproductive state groups. There was no significant difference in any organ weight on any treatment level as compared to the organ weight of the control.

Mortality is listed in Table 86. Most of the deaths were from cannibalism by the more aggressive males. There was no significant difference in mortality between dietary treatment groups for either chemical.

Discussion

In contrast to the subacute test, the chronic study determines whether a small amount of the compound given for a long time differs from the effects of a larger amount of the chemical given for a short time.

Food consumption followed the typical pattern during the egg production period (Figure 25); that is, feed intake was increased during the reproductive period to accomodate for the increase in metabolism and was decreased as production terminated (Scott et al., 1976). Birds show a trend to eat more of a feed that contains less nutrients and less energy.

High levels of any non-nutrient ingredient added to a diet would give less energy per gram of feed. Since birds normally eat to satisfy an energy requirement, they would tend to consume more feed to meet their requirement (Scott et al., 1976).

The pre-egg production feed intake (77.9 to 126 g/b/d) for ducks that weighed about 1200 grams was similar to that reported by Gasaway and Buss (1972) of 36.0 to 73.7 g/b/d for Mallards weighing

Table 80. Effect of feeding DCPD at various levels in the diet for 22 weeks on hemoglobin values of adult Mallard ducks at the end of their first reproductive cycle

Treatment	Level (ppm) in the diet	N	Male Hb gm/dl	N	Female Hb gm/dl	N	Combined[1] Hb gm/dl
DCPD	0	6	11.93	14	12.09	20	$12.05 \pm .266_a$[2]
DCPD	32	6	12.87	14	12.44	20	$12.57 \pm .266_a$
DCPD	100	5	12.20	14	11.90	19	$11.99 \pm .273_a$
DCPD	320	6	12.72	15	11.62	21	$11.94 \pm .259_a$
Total		23	12.44	57	12.01	80	$12.135 \pm .133$

[1]Data reported as treatment mean ± standard error.

[2]Means with the same subscript are not significantly different from their respective control ($P > 0.05$).

Table 81. Effect of feeding DCPD at various levels in the diet for 22 weeks on hematocrit values of adult Mallard ducks at the end of their first reproductive cycle

Treatment	Level (ppm) in the diet	N	Male Hct %	N	Female Hct %	N	Combined[1] Hct T
DCPD	0	6	41.50	15	42.65	21	$42.32 \pm .791_a$[1]
DCPD	32	6	43.33	14	43.86	20	$43.70 \pm .811_a$
DCPD	100	5	42.55	14	43.27	19	$43.08 \pm .832_a$
DCPD	320	6	44.67	15	42.17	21	$42.88 \pm .791_a$
Total		23	43.03	58	42.97	81	$42.98 \pm .399$

[1]Data reported as treatment mean ± standard error.

[2]Means with the same subscript are not significantly different from their respective control ($P > 0.05$).

Table 82. Effect of feeding DCPD at various levels in the diet for 22 weeks on mean corpuscular hemoglobin concentration of adult Mallard ducks (calculated from the data in Table 31 and 32).

Treatment	Level (ppm) in the diet	N	Male MCHC %	N	Female MCHC %	N	Combined[1] MCHC %
DCPD	0	6	28.82	14	28.10	20	$28.32 \pm .331_a$[2]
DCPD	32	6	29.69	14	28.31	20	$28.72 \pm .331_a$
DCPD	100	5	28.71	14	27.61	19	$27.89 \pm .339_a$
DCPD	320	6	28.53	15	27.53	21	$27.82 \pm .323_a$
Total		23	28.95	57	27.88	80	$28.19 \pm .162$

[1]Data reported as treatment mean ± standard error.

[2]Means with the same subscript are not significantly different from their respective control ($P > 0.05$).

Table 83. Effect of feeding DCPD in the diet at various levels for 22 weeks on leukocyte counts of adult Mallard ducks at the end of their first reproductive cycle

Cell	Level DCPD in diet (ppm)	N	Mean[1]	Range
Basophil	0	21	1.48 ± .324[2] $_a$	0-4
	32	20	1.70 ± .332 $_a$	0-5
	100	19	1.79 ± .340 $_a$	0-5
	320	21	1.95 ± .324 $_a$	0-5
Total		81	1.73 ± .165	0-5
Eosinophil	0	21	1.76 ± .440 $_b$	0-6
	32	20	2.25 ± .451 $_b$	0-5
	100	19	2.58 ± .462 $_b$	0-7
	320	21	2.86 ± .440 $_b$	0-9
Total		81	2.36 ± .224	0-9
Heterophil	0	21	23.90 ± 2.86 $_c$	4-57
	32	20	20.30 ± 2.93 $_c$	5-67
	100	19	22.42 ± 3.01 $_c$	8-40
	320	21	27.43 ± 2.86 $_c$	10-61
Total		81	23.58 ± 2.46	4-67
Lymphocyte	0	21	69.00 ± 2.92 $_d$	37-92
	32	20	70.40 ± 2.99 $_d$	25-92
	100	19	69.63 ± 3.07 $_d$	54-82
	320	21	63.47 ± 2.92 $_d$	34-83
Total		81	68.06 ± 1.48	25-92
Monocyte	0	21	3.86 ± .456 $_e$	0-7
	32	20	5.35 ± .467 $_e$	1-11
	100	19	3.58 ± .479 $_e$	0-5
	320	21	4.29 ± .456 $_e$	1-10
Total		81	4.27 ± .232	0-11

[1]Data given as group mean ± standard error.

[2]Means with the same subscript are not significantly different from their respective control ($P > 0.05$).

Table 84. Effect of feeding DCPD at various levels in the diet for 22 weeks on liver and gonad(s) weights in adult Mallard ducks at the end of their first reproductive cycle

Organ	Level of DCPD in diet (ppm)	Mean organ weight (gms)						Organ weight as percent of:					
								Body weight			Brain weight		
		N	M	N	F[1]	N	F[2]	M	F[1]	F[2]	M	F[1]	F[2]
Liver	0	6	26.7[3]	13	31.9[3]	2	36.8[3]	1.87	2.55	2.93	496.4	658.6	739.6
	32	5	25.9[a]	13	29.1[b]	2	43.6[c]	1.96	2.37	3.59	510.6	611.8	887.9
	100	5	33.7[a]	10	30.7[b]	4	44.3[c]	2.49	2.39	3.33	639.0	613.4	936.0
	320	6	25.5[a]	11	37.2[b]	4	43.5[c]	1.88	2.83	3.61	480.2	760.0	917.6
Combined		22	27.8	47	32.1	12	42.7	2.03	2.53	3.40	527.6	659.8	889.1
Gonad(s)	0	6[4]	3.55[3][d]	13	2.48[3][e]	2	31.0[3][f]	0.24	0.19	2.44	66.2	52.0	621.0
	32	5	10.34[d]	13	1.23[e]	2	37.8[f]	0.78	0.10	3.13	207.0	26.0	773.2
	100	5	3.77[d]	10	1.68[e]	4	51.2[f]	0.29	0.13	3.91	72.0	33.2	1094.5
	320	6[5]	9.86[d]	11	1.75[e]	4	30.8[f]	0.75	0.14	2.55	191.0	35.8	651.8
Combined		22	6.86	45	1.79	12	38.8	0.51	0.14	3.08	133.6	37.0	814.5

[1]Females without developing follicles.

[2]Females with developing follicles.

[3]Means with the same subscript are not significantly different from their respective control ($P > 0.05$).

[4]Three out of the five males were in a reproductive state.

[5]One out of the six males was in a reproductive state.

Table 85. Effect of feeding DCPD at various levels in the diet for 22 weeks on organ weights in adult Mallard ducks at the end of their first reproductive cycle

Organ	Level in diet (ppm)	N	Mean organ weight (gms)	Organ weight as percent of: Body weight	Organ weight as percent of: Brain weight
Spleen	0	21	0.669[a][1]	0.053	13.38
	32	20[2]	0.697[a]	0.055	14.24
	100	18	0.753[a]	0.057	14.84
	320	21	0.692[a]	0.054	13.92
Kidney	0	21	8.57[b][1]	0.666	171.52
	32	20	8.68[b]	0.691	179.63
	100	19	8.68[b]	0.662	173.51
	320	21	8.63[b]	0.666	174.29
Pancreas	0	21	4.06[c][1]	0.316	80.93
	32	20	3.56[c]	0.286	74.08
	100	19	3.82[c]	0.291	76.27
	320	21	3.71[c]	0.288	74.90
Proventriculus	0	21	3.97[d][1]	0.308	79.01
	32	20	3.79[d]	0.304	78.70
	100	19	3.94[d]	0.300	78.61
	320	21	4.07[d]	0.313	81.91
Gizzard	0	21	36.53[e][1]	2.82	725.07
	32	20	32.55[e]	2.62	675.16
	100	19	32.61[e]	2.48	646.09
	320	21	32.52[e]	2.48	652.09
Heart	0	21	8.73[f][1]	0.681	174.47
	32	20	8.27[f]	0.667	172.17
	100	19	8.58[f]	0.650	171.07
	320	21	8.89[f]	0.686	179.20
Brain	0	21	5.03[g][1]	--	--
	32	20	4.83[g]	--	--
	100	19	5.03[g]	--	--
	320	21	4.99[g]	--	--

[1] Means with the same subscript are not significantly different from the respective control ($P > 0.05$).

[2] One spleen was lost during the necropsy.

Table 86. Dates of mortality of adult Mallards during the DCPD chronic test, 12/27/76 to 6/2/77

Compound	Level	Sex	Date of:		Cage
			Mortality	Removal	
DCPD	32	F	5/8		11
	100	M	4/2		15
	100	F	5/10		2

about 900 grams. Irby et al. (1967) reported feed consumption of 45 to 68 g/b/d for Mallards weighing about 900 to 1100 grams.

Changes in body weight for DCPD-treated ducks ranged from -2.16 to +0.38 percent of their weight, at the beginning of the test. This change was less than that reported by Gasaway and Buss (1972) for control Mallards of 96 and 104 percent of the animals' weight at the start of their study. These larger changes may have been because of the lighter weight (900 grams) or the fact they only had three birds of each sex. Grandy et al. (1968), using 18-month-old Mallard drakes as controls, reported body weight changes of 8 percent over a 30-day period. Irby et al. (1967) recorded changes in the controls of 14 percent in a 60-day period with 24 ducks of 18 months of age. Changes in body weight while going through a reproductive phase was consistent with normal cycles for birds in that they gained weight for the reproductive period and lost weight at the end, or near the end of their reproductive cycle (Scott et al., 1976).

Total number of eggs laid for all hens on all treatments of DCPD was 2609 in 77 days with an average of 44.2 eggs per hen per season. Normal values range from 28 to 38 eggs per hen per season (Heath et al., 1969; Davison and Sell, 1974; "Federal Register", 1975). The overall increase in egg numbers as compared to previous reports may be due to the fact that every egg was collected as the ducks were in cages rather than outside and/or that the strain of duck used was partially domesticated. Egg production curves followed the normal shape; a sharp rise after initiation of egg production followed by a maintained level of 55 to 75 percent for a few weeks, thereafter declining though not as rapidly as the increase in the beginning (Hafez, 1974).

Eggshell thickness conformed to reports by Heath et al. (1969), Longcore et al. (1971), Heath and Spann (1973), Heinz (1974), Davison and Sell (1974), though their means were slightly lower, ranging from 35 to 39 mm x 10^{-2}. This difference may have been due to a difference in procedure or strain of Mallard used. Exterior shell quality was not affected as no significant numbers of abnormally shaped eggs nor increased numbers of soft shell eggs were noted.

Normal comfort movements noted were the body-shake (körperschutteln), wing-shake (Flugelschutteln), head-shake (köpfshutteln), and wing-flap (Sich-Flugeln) and were in agreement with observations by McKinney (1965; 1975). The body-shake starts with a tail-wag followed by the erection of many body feathers. The shake moves forward on the body to the wings and then head. The wing-shake proceeds as above except there is no head movement and the tail-wag may not occur. The head-shake consists of shaking the bill laterally from side to side. The wing-flap occurs when the bird rises up to its toes slightly and fully opens the wings then flaps them a few times, as in flight.

Sexual behavior also appeared normal, as it was consistent with the findings of Lebret (1961) and Deforges and Wood-Gush (1975a; 1975b; 1976). Pumping of the head in a prelude to mating, social display ("Gesellschaftsspiel") with the head drawn firmly between the shoulders and head feathers erected were noted. Rape (Lebret, 1961; McKinney, 1975; Barach, 1977) was observed by repulsive actions from the harassed female, and is a normal occurrence during the reproduction period in Mallards.

Incubation parameters for the eggs laid by Mallards treated with DCPD are comparable to values given by Prince et al. (1968; 1969b; 1970), Heath et al. (1969), Heath and Spann (1973), Davison and Sell (1974) (see page 63). Greatest mortality during incubation occurred from approximately the 19th day until hatching as was noted by percent dead in shell (Table 78). This high mortality is consistent with the 38 to 66 percent of total mortality for the same reported by Prince et al. (1969a).

Livability of the hatched ducklings raised for two weeks ranged from 98.6 to 99.3 percent (Table 79) and was within the range of normal values of 94 to 99 percent stated in the "Federal Register" (1975).

Hemoglobin gives an indication of the blood's oxygen carrying capacity since one gram of hemoglobin can combine with 1.34 ml of O_2 (Sturkie, 1976). Mean hemoglobin values of drakes treated with DCPD ranged from 11.9 to 12.9 gm/dl. Mean hemoglobin values for hens treated with DCPD ranged from 11.6 to 12.4 gm/dl (Table 80). There values are consistent with other reported values (see page 64). The reported values for the adult Mallard (3 months - 1 year-old Mallard, domestic female duck, and female Pekin and Indian ducks) were in the same range as the DCPD-treated groups of ducks of 9.0 to 15.0 gm/dl.

Hematocrit values give an indication of red blood cell numbers, but the size of the RBC's also influences the packed cell volume. Thus, an increase in RBC numbers with a decrease in size of the cells may make no significant change in the hematocrit value. It was observed that ducks have two sizes of red blood cells which could also give varying results. Mean hematocrit values for the drakes treated with DCPD ranged from 41.5 to 44.67 percent. For the hens treated with DCPD, values ranged from 42.17 to 43.86 percent. These values are comparable to reported values (see page 65). The hematocrit means of DCPD-treated Mallards are comparable to the Mallard values reported by Sturkie (1976) and Hemm and Carlton (1967), while the hematocrit range of ducks treated with DCPD of 35.25 to 51.5 percent was within the range of all reported values.

Though the mean corpuscular hemoglobin concentration (MCHC) is important in the diagnosis of anemic conditions, values for the Mallard have not been reported in the literature. MCHC reflects the overall morphology of the red blood cells (normocytic, macrocytic, or microcytic) being produced by the bone marrow in the animal. This size determination reflects the condition of the bone marrow, metabolic capacity of the red blood cell, and hemoglobin content (Coles,

1974; Sturkie, 1976). One value of MCHC for Mallards of 33.6 percent was reported by Hemm and Carlton (1967), though numbers of animals used were not mentioned. This reported MCHC value is higher than the mean of 28.2 percent for Mallards treated with DCPD. There could be a problem with the interpretation of mean corpuscular values in ducks, because they have two types of red blood cells. One cell type is elongated and narrow with denser chromatin in the nucleus (leptochromatic type) while the other cell type is shorter and rounder with less dense chromatin in the nucleus (pachychromatic type) (Lucas and Jamroz, 1961).

Leukocyte numbers can change with certain chemicals given to an animal. Though a slight change may be a result of a compound, it may be the influence of stress, starvation, or other factors. Comparative differential counts in the literature vary greatly depending on numbers counted, age, physical condition, wild or domestic, and species of duck. Some values reported for ducks are given on page 66. The duck values cited in Sturkie (1976) had the closest leukocyte count in comparison to the Mallards treated with DCPD while the other authors cited indicated a higher heterophil count. There were more lymphocytes than heterophils in the DCPD-treated ducks, which is generally true for most avian species (Sturkie, 1976). DCPD-treated ducks' differential counts showed extreme ranges which were consistent with reported values, shown on page 66.

There is generally some difficulty in differentiating eosinophils from heterophils in the duck (Hemm and Carlton, 1967). The features used to distinguish between them for the differential counts on DCPD-treated ducks were: (1) heterophil's nucleus stains fainter or with more variability than the eosinophil's, (2) heterophil's cytoplasm is clear while the eosinophil has a light blue cytoplasm and, (3) the heterophil's granules are characteristically rod shaped while eosinophil's granules are characteristically round. The whole area of duck hematology, especially differential counts and mean corpuscular values, needs much additional work so that correct interpretations can be made.

Individual organ weights can give an indication of pathologic changes occurring in that organ; especially hypertrophy, hyperplasia, and atrophy. All organs from the treated ducks appeared normal at the time of sacrifice, except that some of the spleens showed discoloration in a number of the controls and those on treatment. No trends in appearance or weight difference were noted for any other organ. All organs were normal in weight as is noted when compared to the controls and other reported values (see page 67).

The Pekin's organ weights, expressed as a percent of body weight, were consistently twice the Mallards, while the 15-week-old Mallards were similar to the DCPD-treated ducks except for the kidney. The controls were consistent with the treatment groups except for the male gonads, because there were some males still in a reproductive state in the treatment groups and not in the control group.

Conclusions

Oral LD_{50}: DCPD is relatively harmless to Mallards. An LD_{50} could not be determined when levels as high as 40000 mg/kg were administered.

Oral LC_{50}: Mallards fed DCPD reached zero (essentially) feed consumption at about 70000 ppm but at no level (highest level 90000 ppm) did mortality exceed 30 percent. Thus, they probably could not ingest enough chemical to reach an LD_{50}.

Oral Chronic: There was no effect in the parameters measured, which included body weight, cracked eggs, incubation parameters, normal ducklings, 14-week-old survivors, eggshell thickness, teratogenicity, behavior, gross pathology, histopathology, blood parameters, and mortality, of adult Mallards fed DCPD.

Toxicity of DCPD to Bobwhite Quail

TEST 1 - ACUTE (LD_{50})

The research was conducted to determine the lethal dose of DCPD for 50% of the test subjects (LD_{50}), the lethal dietary concentration of DCPD for 50% of the test subjects (LC_{50}), and the chronic toxicity of DCPD to Bobwhite quail (Colinus virginianus). The tests were conducted in a windowless house at the Michigan State University Poultry Science Research and Teaching Center. The Bobwhites were procured from the Poultry Science Department, Michigan State University, East Lansing, MI 48824.

Procedure

This test was designed to determine the single, 14-day, oral dose LD_{50} of DCPD to the Bobwhite.

Adult Bobwhites, approximately one year of age, in non-laying condition, were utilized. The birds were maintained indoors in cages measuring 85.1 cm (l) x 89 cm (w) x 24.1 cm (h); 20 birds per cage. Cage floor space per bird was 379 cm^2.

Body weights of all birds were recorded succeeding a one-week holding period. A two-week acclimatization period followed. Body weights were again recorded at the termination of acclimatization to note any significant weight loss before range finding was initiated.

Preliminary range finding was conducted to establish the approximate lethal dose. A series of dosages was employed for the test to give a mortality range of 10 to 90 percent.

Testing

Birds used for testing were maintained on a quail breeder diet (Appendix G: Composition of Feed). The feed was free of antibiotics and medication. Feed and water were provided ad libitum throughout the testing period with the exception of a 15-hour minimum fasting period before oral administration of test chemical. Weekly feed consumption was determined for each group.

The initial DCPD test utilized twenty birds, ten of each sex, per dose level. The additional DCPD test utilized 10 birds, five of each sex. Weights were recorded immediately preceding the dosing, and on the third, seventh, and fourteenth days of the succeeding two-week observational period. Post-treatment behavior was observed for one hour immediately following dosing, again at 4-5 hours, and daily thereafter for the duration of the observational period.

Administration was by drenching per os from a 1 cc syringe with a length of polyethylene tubing (0.762 mm ID and 1.29 mm OD) attached to a 22 ga. needle. The length of tubing corresponded with the distance from the back of the oral cavity to the eosphageal opening of the proventriculus. This insured a uniform location for the introduction of the test substance.

Necropsies were performed on all birds, including controls, at the time of death or termination of the observational period.

Statistical Analysis

The LD_{50} was analyzed by the method of Litchfield and Wilcoxon (1949). Weight changes were analyzed by least squares analysis of covariance with log transformation and the two-sided Dunnett t-test with modification for unequal replication. Feed consumption data were not appropriate for meaningful statistical analysis.

Results

Range finding pilot studies were conducted to provide a practical dosage span to be used in the acute test.

DCPD range finding began at 400 mg/kg body weight. The dose was repeatedly doubled until a level of 3200 mg/kg body weight was reached, with deaths occurring at 1600 mg/kg body weight and 3200 mg/kg body weight. Three additional trials were conducted to provide more reliable data to use in the determination of the LD_{50} dose levels. Overall results are shown in Table 87.

Mortality for the quail treated *per os* with DCPD is listed in Table 88. The acute oral LD_{50}, determined by the method of Litchfield and Wilcoxon (1949), was 1010 mg/kg with a 95% confidence interval of 933.2 - 1093.1 mg/kg.

The mortality curve of DCPD for the Bobwhite is plotted in Figure 27. Most deaths occurred within 48 to 96 hours after dosing with DCPD. There was no mortality nor clinical sign differences between the sexes among the treated groups.

Responses of quail to DCPD dosing were noted after 24 hours when activity decreased and the birds became quiescent. Those that attempted to walk were unsteady and lacked coordination. Recovery or coma and death followed by 96 hours post-treatment.

During the 14-day post-treatment period, no further signs of intoxication nor significant weight changes from the control were noted in the treated birds (see Table 89).

Necropsies of all birds that died and those that were sacrificed at the end of the post-treatment period showed no gross pathological changes.

Feed consumption, for the 14-day post-treatment period, is listed in Table 90.

Quail treated with DCPD at levels higher than 200 and 400 mg/kg showed depressed feed consumption, with a marked decrease at the 1400 and 1600 mg/kg levels, during the first week. By the second week feed consumption had improved at all levels.

Table 87. Results of DCPD LD_{50} range finding trials

Chemical	Level of DCPD (mg/kg body weight)	Number of birds	Mortality %
DCPD	400	1	0
	800	1	0
	1000	1	0
	1100	3	67
	1200	3	100
	1300	3	100
	1400	3	100
	1500	3	67
	1600	4	100
	3200	3	100

Table 88. Mortality of adult Bobwhite quail during a 14-day period following a single per os dosing with DCPD.

Treatment level (mg/kg)	Mortality No. died/No. treated male	female	Combined (%)
0 (control)	0/15	0/15	0
200	0/5	2/5	20
400	1/5	1/5*	10
600	1/5	0/5	10
800	5/15	4/15	30
900	4/10	1/10	25
1000	10/15	9/15	63
1100	2/10	4/10	30
1200	11/15	9/15	61
1400	5/5	4/5	90
1600	9/10	----	90

* Accidental death

Figure 27. Percent mortality of adult Bobwhites (equal numbers of each sex) given a single per os dose of DCPD and observed for 14-days post-treatment. In the regression equation x = dose of DCPD in mg/kg of body weight and y = percent mortality.

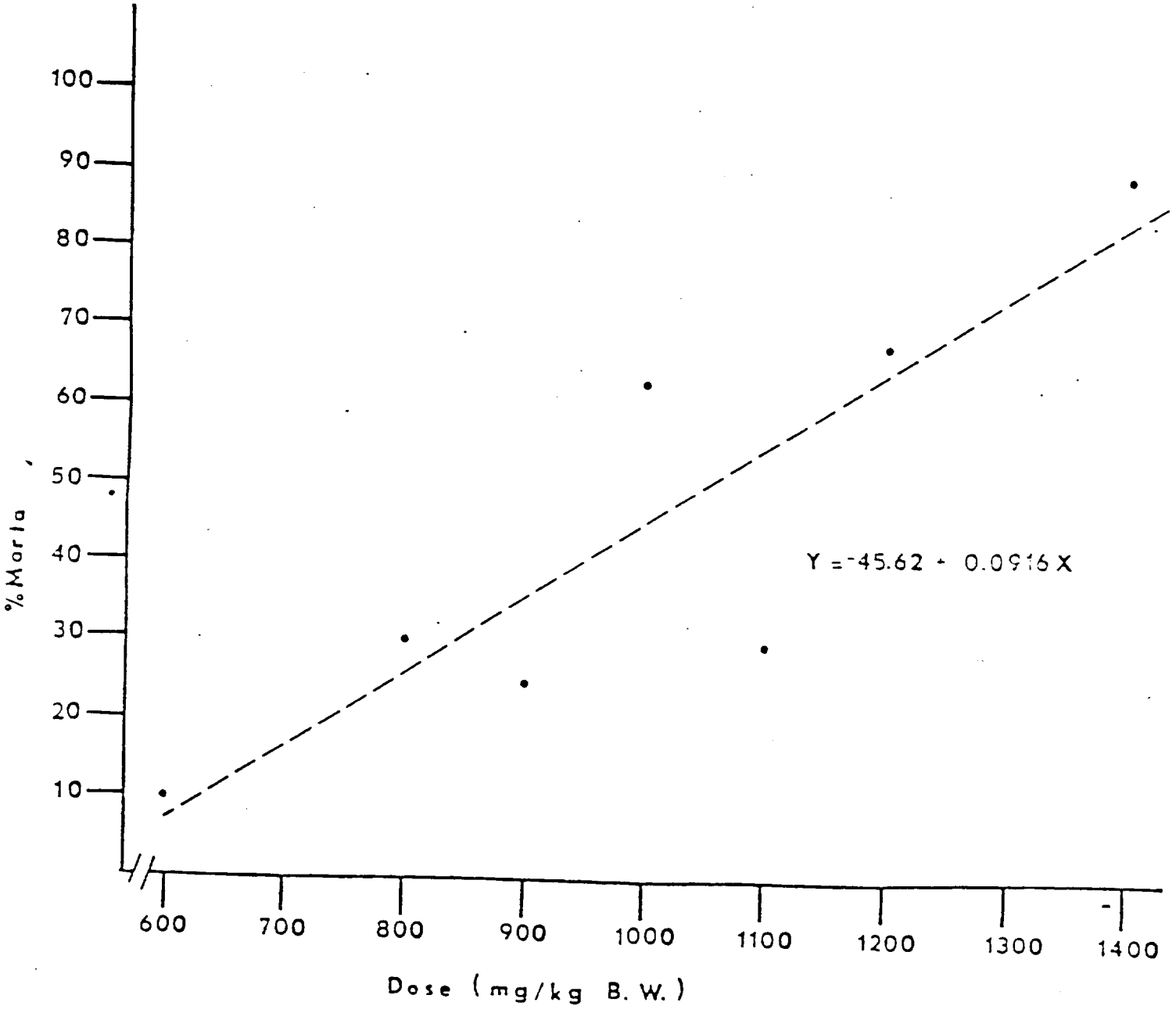

Table 89 Quail body weight changes during post-treatment for LD_{50} (mean values)

DCPD level (mg/kg)	n	Mean body weight (g) Day 0	Day 14	Mean change (g/b/d)
0	19	205.74	198.74	$-.500_{a1}$
200	8	188.75	196.38	$+.545_{a}$
400	8	189.00	178.88	$-.742_{a}$
600	9	186.44	182.11	$-.309_{a}$
800	21	184.71	193.00	$+.592_{a}$
900	15	201.80	198.73	$-.219_{a}$
1000	11	207.45	191.18	-1.162_{a}
1100	14	183.93	192.29	$+.240_{a}$
1200	10	197.70	191.80	$-.421_{a}$
1400	1	194.00	189.00	$-.357_{a}$
1600	1	205.00	198.00	$-.500_{a}$

[1] Means having the same subscript are not significantly different from their respective control ($P>0.05$).

Table 90. Quail feed consumption (g/b/d) during post-treatment for LD_{50}

DCPD level (mg/kg)	n	Days 0-7	Days 8-14
0	19	10.26	13.60
200	8	11.04	11.17
400	9	9.42	9.04
600	9	6.29	11.35
800	21	6.25	10.14
900	15	7.65	13.19
1000	11	8.09	12.57
1100	14	7.65	15.21
1200	10	8.01	14.44
1400	1	3.36	13.57
1600	1	1.36	12.40

Discussion

The LD_{50} of DCPD for rats and mice (Hart and Dacre, 1977) was smaller than the LD_{50} of DCPD for Bobwhites. Quail were roughly twice as resistant to DCPD as were rats and approximately five times as resistant as were mice. Bobwhites were, however, less resistant to DCPD than were Mallard ducks and mink as reported in this study.

Table 25 lists several compounds and their LD_{50} values for the Bobwhite (Tucker and Crabtree, 1970). The LD_{50} of DCPD for the Bobwhite is included in the table for comparison of relative toxicities. Based on the chart on page 29, DCPD is slightly toxic to the Bobwhite.

The slope of the dose-response curve is an estimate of the margin of safety of a compound (the magnitude of the range of doses, and thus responses, between a no effect dose and a lethal dose). A steep curve limits the range of doses. A flat curve encompasses a large range of doses. The dose-response curve of DCPD for the Bobwhite was somewhat flat (Slope = .096) indicating variable response to the compound.

When not lethal, DCPD did not produce lasting effects on feed consumption and/or body weight during the 14-day observation period. The feed consumption of Bobwhites post-DCPD treatment in this study followed the same feed consumption pattern as Mallard ducks dosed with DCPD. Generally, a decrease in feed consumption during the first week post-treatment was succeeded by an increase in feed consumption, up to control levels, the second week. Reduced post-treatment feed consumption is not an uncommon result in the reported literature. Coburn and Treichler (1946), and Dahlen and Haugen (1954) reported reduced feed consumption in the Bobwhite following treatment with DDT, aldrin, dieldrin, toxaphene, and lindane, respectively.

During the 14-day post-treatment period, the body weight change of DCPD-treated birds showed no significant difference from the body weight change of the control birds; a maximum of 0.5 percent change. Results from other investigators varied. Dahlen and Haugen (1954) reported an average weight loss of 15 to 25 percent in Bobwhites dosed with aldrin, dieldrin, toxaphene, or lindane. Bergstrand and Klimstra (1962) reported a mean weight gain of 3.5 percent in Bobwhites dosed with fenuron. Kinkead et al. (1971) conducted mammalian studies with DCPD and found normal weight gains in the treated animals. As reported elsewhere in this study, a six percent mean increase in the body weight was observed in Mallard ducks dosed with DCPD.

The similarity in response to DCPD administration of male and female Bobwhites is consistent with findings on other compounds investigated by Coburn and Treichler (1946); Dahlen and Haugen (1954); and Tucker and Haegele (1971). Hart and Dacre (1977) reported no difference in response, attributable to sex, in either rats or mice dosed with DCPD.

The majority of the deaths of Bobwhites dosed with DCPD occurred within 48 hours of the treatment. Of that majority, half occurred on day 1 and the other half occurred on day 2.

The remaining deaths occurred sporadically. One to two day mortality time was reported by Coburn and Treichler (1954) after dosing quail with DDT.

TEST 2 - SUBACUTE (LD_{50})

Procedure

This test was conducted to determine the minimum repeated oral dosage (mg/kg/day) of DCPD that was lethal to Bobwhite chicks.

A range finding pilot study was conducted with DCPD to determine the effect on mortality, feed consumption, and body weight. Since the mortality that did occur appeared unrelated to the dietary levels of DCPD, a series of dosages was utilized to determine the point of feed refusal instead of 50 percent mortality.

Testing

Randomly selected day-old Bobwhite chicks were housed indoors in a Petersime Brood Unit[2] and maintained on a standard quail starter diet (Appendix G: Composition of Feed); free of antibiotics and medication. Feed and water were provided ad libitum. At 14 days of age the chicks were segregated into groups of ten birds of undetermined sex. Each group of birds was randomly assigned to one of ten dietary treatments. During the eight-day test period, treated feed was fed for the first five days and untreated feed was fed for the remaining three days. Feed and water were provided ad libitum throughout the test period.

Levels of DCPD employed for the subacute test were partially determined by the LD_{50} value, the slope of the dosage-mortality curve, the variation within a group's response to the same dose, and the results of the range finding pilot studies.

The eight-day range finding pilot test utilized six birds for each dietary treatment per chemical. Dietary treatments consisted of 4000, 8000, and 16000 ppm. Treated feed was fed for the initial five days of the test and untreated feed for the remaining three days. The three-day (untreated feed) period was included to avoid overestimation of the lethal dosage by calculating mortality before the compound had sufficient time to act.

Body weights were measured at the initiation of the test, the transition between feeding treated and untreated feed, and the

---

[2] Petersime Incubator Company, Gettysburg, OH 45328

termination of the test. Feed consumption was estimated by providing a known amount of treated or untreated feed for the birds and weighing the remainder on days five and eight of the test, respectively.

Results of the range-finding test were:

DCPD level in diet (ppm)	Mean change in body wt. (g/b/d)	Mean feed consumption (g/b/d)	Mortality (%)
4000	+2.315	7.085	0
8000	+0.965	3.740	66.7
16000	+1.785	2.400	0

Since the mortality was greater than 50 percent (66.7 percent at the 8000 ppm level) in the range finding study, the subacute levels were set below two percent of the diet.

The test diets were prepared by dissolving the chemical in corn oil and hand mixing with quail starter feed to make a premix. The premix was then added to a standard quail diet to yield the appropriate dietary level (see Appendix H: Diet Preparation). The DCPD-treated diets' chemical-corn oil solution constant was two percent of the diet. The control diet consisted of two parts corn oil to 98 parts feed by weight. The ten dietary treatments used for testing the chemical were as follows: DCPD (ppm): 0, 2000, 4000, 6000, 8000, 10000, 12000, 14000, 16000, and 18000.

Body weights were recorded on days zero, five, and eight of the test period. Feed was weighed on days zero and five (treated feed) and days six and eight (untreated feed) to provide estimates of average feed consumption. Observations on feed wastage were taken into account in determining the estimated point of zero feed consumption.

Any signs of intoxication or abnormal behavior during the test period were noted. All birds that died during the trial and those that survived until the termination of the experiment, were necropsied.

Statistical Analysis

Slopes of values for feed consumption, body weight change, and predicted zero feed consumption were determined by regression analysis.

Results

Compared to the control group, feed consumption of the chicks on diets that contained DCPD increased in six of the treated groups and decreased in three of the treated groups (Figure 28). The

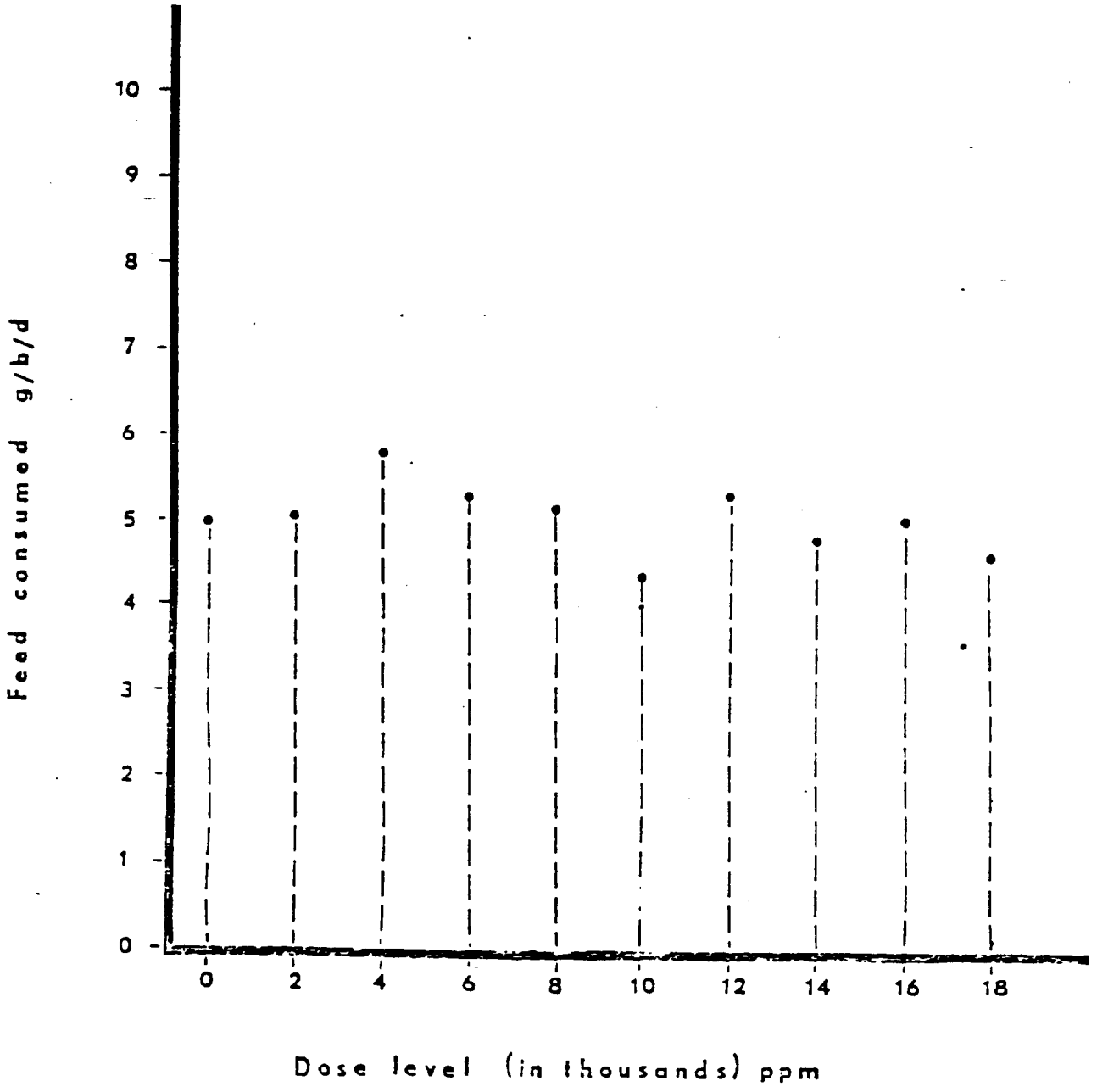

Figure 28. The effect of feeding DCPD at various levels in the diet for five days on feed consumption of 14-day-old Bobwhite quail chicks.

differences ranged from a 12.2 percent decline (0.16 g/b/d less than the control) for the birds that received the 10,000 ppm diet to a 16.4 percent increase (1.82 g/b/d more than the control) for the birds that received the 4,000 ppm diet, with a mean 1.4 percent (0.07 g/b/d) above that of the control. The equation describing the regression line depicting feed consumption was y = 5.343 - 0.00003x (Figure 29) with a correlation between feed consumption and level of DCPD in the diet of -0.4683. The predicted zero feed consumption was calculated from this line to be 73028 ppm DCPD in the diet.

Body weight data (Figure 30) showed that all treatment groups gained weight. Total intake of DCPD ranged from 357.4 to 3051.3 mg/kg/day with the least amount of intake in the three lowest level groups (2000, 4000, and 6000 ppm). Quail fed the lower level diets (2000 - 8000 ppm) showed a mean gain of 2.86 g/b/d, a 0.1 g/b/d decrease as compared to the control. Birds on higher levels (10000 - 18000 ppm) showed a mean gain of 2.38 g/b/d, a 0.58 g/b/d decrease from the control. The slope of the regression line depicting body weight changes was -0.00004, (Figure 31) with a correlation between the level of DCPD in the diet and weight gain of -0.6812. Predicted zero body weight gain was calculated to be 80108 ppm DCPD in the diet.

There were no trends in mortality (Table 91). Both the 2000 and 10000 ppm groups had the highest mortality at 20 percent. The 18000 ppm group, which had the highest intake of DCPD, had 10 percent mortality. All other groups had no mortality even though levels of 6000 ppm and higher had DCPD intake levels above the LD_{50} value of 1010 mg/kg body weight. Correlation between mortality and mg DCPD/kg/day ingested was -0.0648.

During the three-day post-treatment period, all groups, except the 10000 ppm group, had increased feed consumption as compared to the control (Figure 32). There were no trends in feed consumption since the slope of the regression line was +.00001 and the correlation between the level of DCPD in the treated diets and feed consumption was +0.1486. The increases in feed consumption ranged from 3 percent, at the 4000 ppm level, to 19.94 percent, at 8000 ppm level, with a mean increase of 7.74 percent (5.16 g/b/d) as compared to the control.

Body weight changes (Table 92) during the post-treatment period showed no trends. The slope of the regression line was +0.00004, and the correlation between the level of DCPD in the treated diets and feed consumption was +0.3730. All groups, with the exception of the 8000 ppm group, showed gains ranging from 4.83 g/b/d, at 16000 ppm level, to 3.37 g/b/d, at 14000 ppm level, with a mean gain of 3.80 g/b/d. This was only 0.718 g/b/d greater than the control. At the 8000 ppm level, the body weight gain was 2.6 g/b/d which was 0.44 g/b/d less than the control.

No gross pathological changes between the DCPD-treated groups and the control were observed during necropsies.

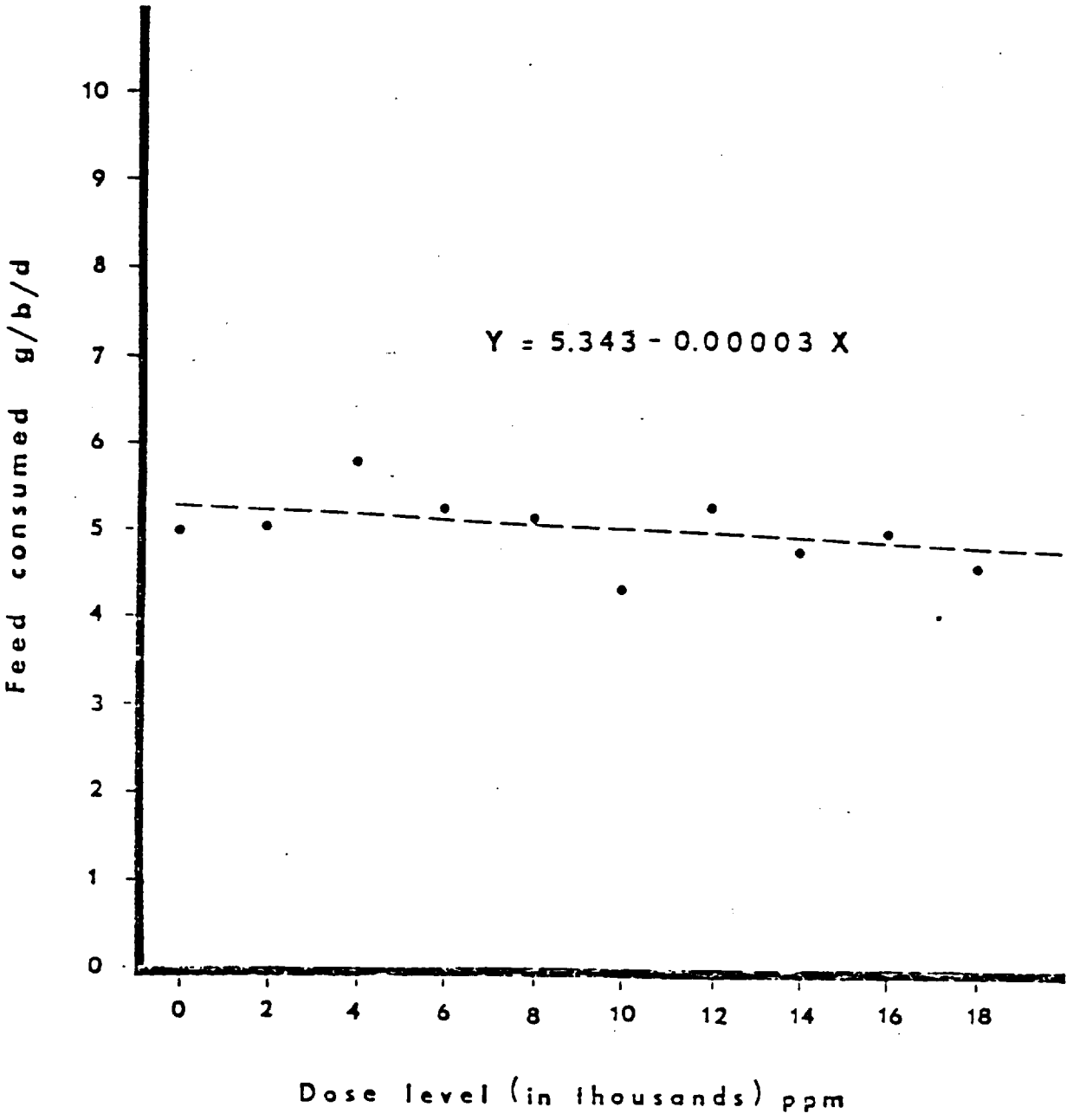

Figure 29. The regression equation of the data shown in Figure 28. In the regression equation x = ppm of DCPD in the diet and y = feed consumption (g/b/d).

Figure 30. Effect of feeding various levels of DCPD in the diet for five days on body weight change of 14-day-old Bobwhite chicks.

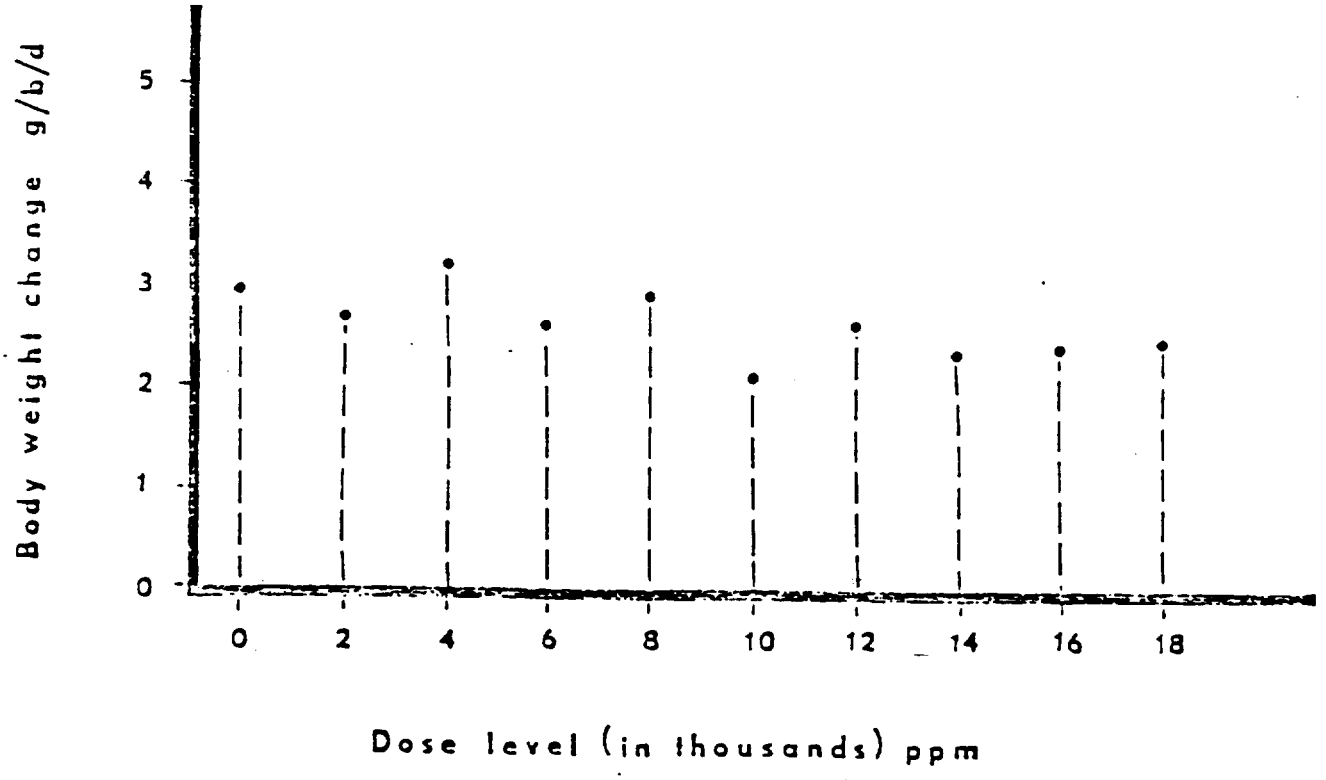

Figure 31. Regression equation of the data shown in Figure 30. In the regression equation x = ppm DCPD and y = body weight change in g/b/d.

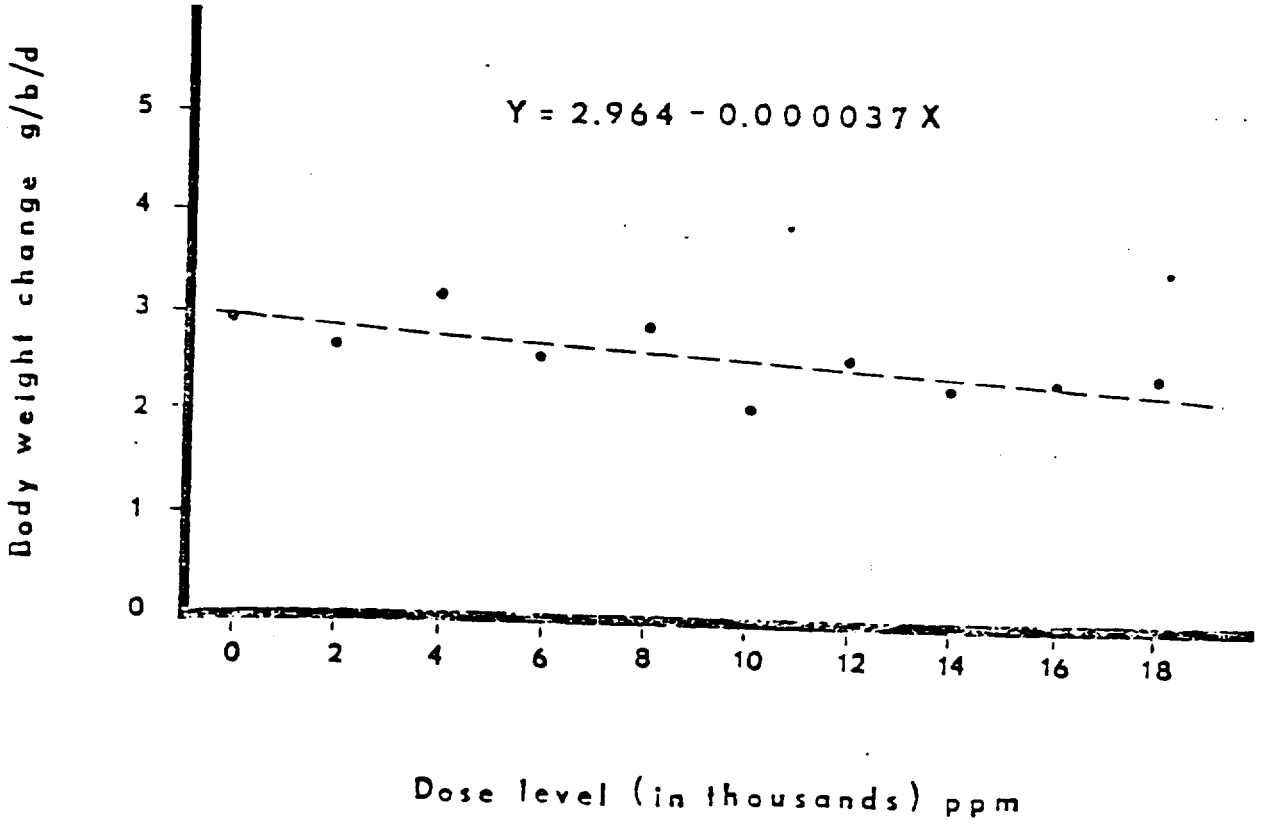

Table 91. Calculated DCPD intake over 5 days and mortality over 8 days for 14-day-old Bobwhite chicks on LC_{50} trial.

DCPD level in diet (ppm)	DCPD consumed/ day (mg)	Mean body wt.[1] (gms)	DCPD consumed (mg/kg/day)	Mortality (%)
0	0	28.3	0	0
2000	10.4	29.1	357.4	20
4000	23.3	30.9	754.0	0
6000	31.8	28.0	1135.7	0
8000	41.3	30.0	1376.7	0
10000	43.9	25.7	1708.2	20
12000	63.8	30.1	2119.6	0
14000	67.2	26.2	2564.9	0
16000	80.6	28.1	2868.3	0
18000	83.3	27.3	3051.3	10

[1] Mean body weight of treatment group for five-day interval.

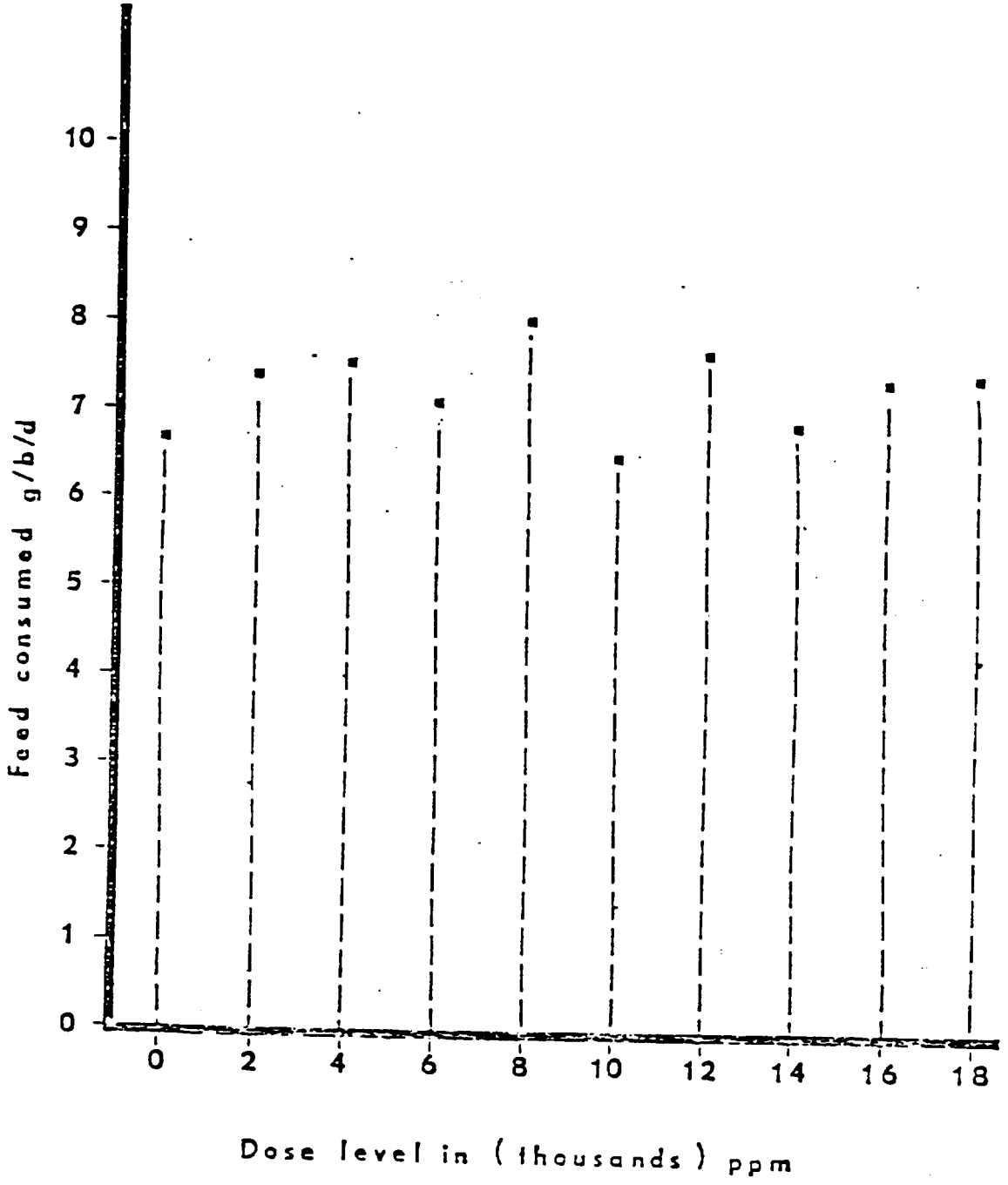

Figure 32. Feed consumption of Bobwhite chicks fed untreated feed during three-day post-treatment period after withdrawal of DCPD-treated diets.

Table 92. Body weight change of Bobwhite chicks during the 3-day period after withdrawal of DCPD-treated diets.

DCPD level in diet (ppm)	Weight change (g/b/d)	Feed consumed/ weight change
0	3.04	2.19
2000	3.50	2.11
4000	3.70	2.04
6000	3.90	1.82
8000	2.60	3.08
10000	3.96	1.64
12000	3.67	2.09
14000	3.37	2.04
16000	4.83	1.53
18000	3.45	2.16

Discussion

LC_{50} values of DCPD could not be determined for the Bobwhite due to insufficient mortality, even though the average mg of compound consumed per bird per day was greater than the LD_{50} value. The mortality occurring in the DCPD-fed birds reached a maximum of only 20% and was not dose related. The predicted point of zero feed consumption was about 70000 ppm. These results are in agreement with the reported undeterminable LC_{50} value for the Mallard duck fed DCPD-treated diets and predicted point of zero feed consumption of 77300.

Values taken from LC_{50} determinations (Heath et al., 1972) of 98 pesticidal chemicals are listed in Table 28. The LC_{50} value of DDT in Table 28 was taken from results by Heath and Stickel (1965).

In general, the feeding of DCPD-treated diets to the Bobwhite had little effect on their feed consumption, although small decreases in feed consumption at the higher DCPD-dietary levels were noted. This mild reduction of feed intake may have been due to a repellant effect of the compound rather than a toxic effect. Voluntary feed restriction of treated diets is not uncommon. Ernst (1966) reported that quail voluntarily restricted their feed intake when sufficient levels of some pesticides were added to their diets. Frings and Boyd (1952) reported olfactory discrimination by the Bobwhite. Body weight gains were generally reduced in Bobwhites fed DCPD; the least weight gains occurred in birds fed the highest dietary levels. However, the decreased weight gain of the birds fed DCPD-treated diets was more pronounced than the reduction in feed consumption.

Feed efficiency of Bobwhites fed DCPD-treated diets during the three-day post-treatment period showed no trends.

All birds on dietary levels of DCPD, other than the two lowest levels, consumed a greater amount of chemical (mg/kg/day) than the LD_{50} value. Fitshugh and Schouboe (1965) reported that animals tolerating an amount of chemical in their diet greater than the LD_{50} value was uncommon. A possible explanation of the phenomena investigated by Fitshugh and Schouboe (1965) is an observation by Stickel et al. (1965) who reported that absorption of some compounds through the gastrointestinal wall can be more efficient if the compound is incorporated into the diet than when given as a single dose. Heath et al. (1972) reported that exposure of a compound via the diet is often gradual, allowing sufficient time for the degradation of unstable compounds, but not necessarily the stable compounds.

TEST 3 - CHRONIC

Procedure

This test was designed to determine the effects of continuous long term exposure of DCPD to the adult Bobwhite throughout a single reproductive cycle.

The test consisted of four dietary treatment groups, three treated levels (400, 1250, and 4000 ppm) plus a control. Each treatment group consisted on one female and one male housed in a single cage replicated fifteen times. The birds were allowed a two-week acclimatization period before the initiation of the test.

Testing

Test diets were prepared by the addition of a premix to a standard quail breeder feed to attain the appropriate dietary levels of DCPD (Appendix H: Diet Preparation). The control diet consisted of two parts corn oil to 98 parts feed by weight. The diets were fed to the birds for a minimum of ten weeks before the initiation of egg production and a minimum of ten weeks after the attainment of 50 percent egg production. Feed and water were provided ad libitum throughout the test.

Feed consumption was measured biweekly for the duration of the experiment. Body weights were measured at 0, 2, 4, 6, and 8 weeks and at the termination of the study. Body weights were not measured during egg production to avoid any adverse effects that handling may have had on egg production.

During the pre-egg production period (Nov. 1 to Jan. 8), the testing room was maintained at approximately 18°C with six hours of light provided per day. To induce egg production, the lighting schedule was increased to 16 hours of light per day. This schedule was maintained throughout the production period (Jan. 8 to May 28). Temperature of the test room during the production period ranged from 15°C to 28°C.

Egg production, mortality, morbidity, and any observable clinical signs of intoxication were recorded daily. All birds that died during the study, were subjected to gross necropsy. Hemoglobin concentration, packed red cell volume (hematocrit value), and differential counts were determined for all surviving birds at the termination of the test.

Egg Collection, Storage, and Incubation

Each day, eggs were collected, marked with the corresponding cage number and date, and stored at 12.8 to 15.6°C until placed in an incubator. The storage time ranged from zero to six days.

Eggs were set at weekly intervals in a Jamesway single stage Model 252 incubator[3]. The incubator was maintained at an average internal temperature of 37.5°C (range 36.9 to 38.1°C) and average relative humidity of 56 percent (range 52 to 65 percent). All eggs were candled on day 0 for shell cracks and again on day 14 to determine fertility and/or early embryonic death. Eggs that were cracked,

---

[3] James Manufacturing Company, Inc. (a subsidiary of Butler Manufacturing Co.), Fort Atkinson, WI 53538

infertile or that contained early deads were removed and disposed of. Fertile, developing eggs were put in pedigree hatching baskets and were transferred to a hatching unit (Jamesway Model 252) on day 21. The average temperature and relative humidity of the hatcher was 37.2°C (range 36.8 to 38.1°C) and 67 percent (range 65 to 70 percent), respectively. On day 24 the hatched chicks were removed from the hatcher, wing banded, and housed in a Petersime Brood Unit for a two week observational period. Untreated feed and water were provided ad libitum during the two weeks. Mortality was recorded daily. Survivors were weighed and sacrificed at the termination of the two week observational period and livability calculated.

Eggs that did not hatch were broken open, examined, and recorded in one of the following categories; pipped live, pipped dead, live in shell, or dead in shell.

Eggs from one day's production were collected at biweekly intervals to be measured for eggshell thickness. The eggs collected for shell measurement were cracked open at the girth, the contents washed out, and the shells air dried for a minimum of 48 hours. Measurements of the thickness of the shell plus the membranes were taken at four points around the girth using a micrometer[4] calibrated to 0.01 mm units.

Histopathology

At the termination of the test all surviving animals were killed by cervical dislocation, a gross examination of the carcasses performed, and the organs (liver, spleen, kidney, pancreas, proventriculus, gizzard, heart, and brain) excised and weighed. A sample of these organs plus lungs, adrenals, duodenum, and sciatic nerve were then placed in ten percent neutral buffered formaldehyde (Luna, 1968) and prepared for histopathologic examination according to routine procedures, as described in Appendix C.

Hematological Preparation

Determinations of differential counts, packed red cell volume, and hemoglobin concentration were completed on all birds that survived until the termination of the experiment (see Appendix D, E, and F).

Statistical Analysis

Data from the chronic study were treated statistically by analysis of variance; sample units, with three exceptions, for the variables measured were the individual cages. The exceptions were

---

[4] Federal Products Corporation (a subsidiary of Esterline Corp.) 1144 Eddy Street, Providence, RI

for body weight change, organ weight, and hematological parameters where the sample units were the individual birds.

Dunnett's t-test (with modification for unequal replication where applicable) was used to compare all treatment groups to the control for each variable, except percent livability of progeny. The latter was analyzed by the split-plot design (Gill, 1978) with arcsin transformation.

Results

Dietary levels of the test substances were determined from the results of the LD_{50} experiment and consultation with the Project Officer (United States Army Medical Command). Normal feed consumption and body weight gains at 4000 ppm DCPD coupled with mortality at the 10000 and 18000 ppm DCPD levels were used in the determination of the DCPD-dietary levels of 0, 400, 1250, and 4000 ppm used in the chronic test. Dietary intake of the test substances was expressed as ppm as opposed to mg/kg/day.

Feed consumption data for adult Bobwhites fed DCPD-treated diets or control diet are presented in Figure 33. Each point plotted is the mean of 15 cages, each housing a male and female bird. There was no significant difference in feed consumption between birds fed DCPD diets and birds fed a control diet. A general increase, over the entire test period, in feed consumption was noted on all dietary levels.

Body weight change data of Bobwhites fed DCPD-treated or control diets for the initial 10 weeks of the test period are presented in Table 93. During the 10-week period no significant differences in body weight change were found between birds fed the treated diets and the control birds.

Body weight change was measured again at the commencement of egg production, and at the termination of the test. These data, catagorized according to sex, are given in Table 94. No significant differences between the mean body weight change of treated groups and the control group were found for either sex.

Mortality data of Bobwhites fed DCPD or the control diet are presented in Table 95. No diet related trends were noted. The majority of the deaths occurred during a 48-hour period at approximately the 62nd day of the test. The cause of death of the twelve birds which died during the 48-hour period could not be determined. Other mortality was sporadic.

Egg production data for the DCPD-study Bobwhites are plotted in Figure 34. Each point plotted is the mean of 15 cages of one hen each. Percent production was based on hen-day production. Analysis of the data revealed that the egg production of the hens fed 400 ppm DCPD was significantly less than the egg production of the hens fed a control diet. No significant difference between the egg production

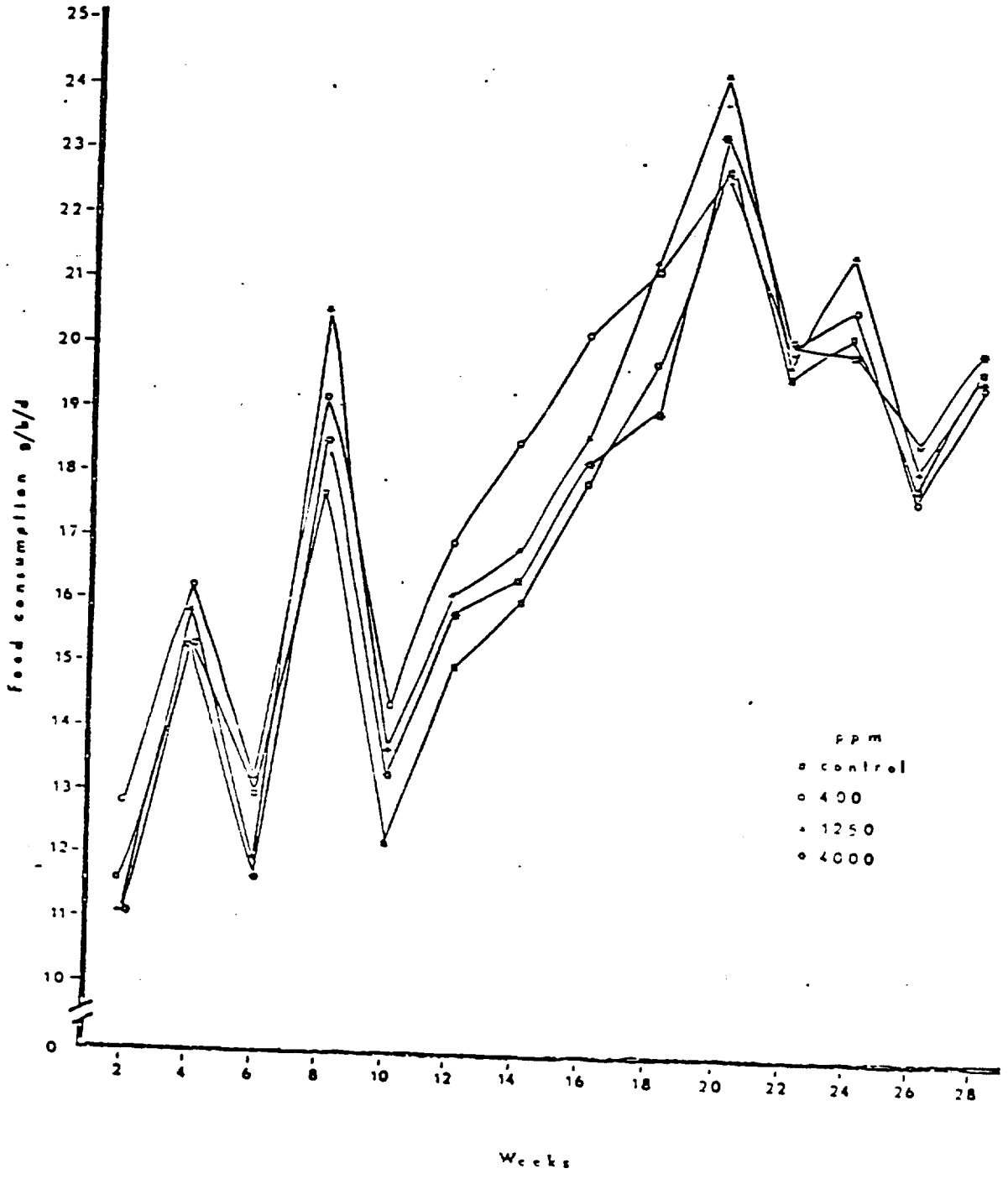

Figure 33. Effect of feeding various levels of DCPD in the diet for 28 weeks on feed consumption of adult Bobwhites. Each point represents the mean of fifteen females.

Table 93. Effect of feeding DCPD at various levels on body weight changes of Bobwhites for the 10 weeks prior to the onset of egg production.

DCPD level (ppm)	n	Biweekly body weight change (%)				
		2 weeks	4 weeks	6 weeks	8 weeks	10 weeks
0	30	- 6.46$_a$[2] (±3.47)	+12.63$_a$[2] (±4.86)	+ 0.56$_a$[2] (±3.67)	+ 5.16$_a$[2] (±2.88)	- 2.14$_a$[2] (±2.13)
400	30	- 4.50$_a$ (±5.28)	+12.29$_a$ (±5.93	- 2.78$_a$ (±4.82)	+ 8.55$_a$ (±6.79)	- 1.59$_a$ (±2.17)
1250	30	- 8.56$_a$ (±4.25)	+12.06$_a$ (±19.70)	- 0.84$_a$ (±3.76)	+ 8.75$_a$ (±4.08)	- 1.69$_a$ (±2.39)
4000	30	- 5.51$_a$ (±3.34)	+ 7.73$_a$ (±18.88)	- 2.65$_a$ (5.49)	+ 9.48$_a$ (±4.22)	- 0.83$_a$ (±2.32)

[1] Data reported as mean ± standard deviation.

[2] Numbers with the same subscript are not significantly different from their respective control ($P>0.05$).

Table 94. Effect of feeding DCPD at various levels for 28 weeks on body weight change of Bobwhites during the 10-week reproductive period.

Sex	DCPD level (ppm)	n	Mean body weight: Pre-production (week 18)	Mean body weight: Termination (week 28)	Body weight change (%)[1]
Female	0	12	201.58	220.00	+ 9.21 ± 6.76$_a$[2]
	400	13	198.00	217.08	+ 9.64 ± 13.85$_a$
	1250	14	205.43	215.21	+ 5.83 ± 14.51$_a$
	4000	13	201.38	218.54	+ 8.69 ± 6.11$_a$
Male	0	13	200.15	201.08	+ 0.42 ± 6.53$_b$[2]
	400	12	200.00	198.14	- 0.91 ± 2.89$_b$
	1250	13	201.85	222.08	+ 1.39 ± 5.89$_b$
	4000	12	199.92	195.08	- 2.31 ± 5.10$_b$

[1] Data reported as mean ± standard deviation.

[2] Means having the same subscript are not significantly different from their respective controls ($P>0.05$).

Table 95. Effect of feeding DCPD on mortality of Bobwhites during the 28-week chronic study.

DCPD level (ppm)	Mortality[1] (days 61-63)	Total mortality	Mortality (%)
0	4	5/30	16.67
400	2	3/30	10.0
1250	2	3/30	10.0
4000	4	5/30	16.67

[1] The majority of deaths occurred during a 48-hour period at approximately the 62nd day of the test (see text for description).

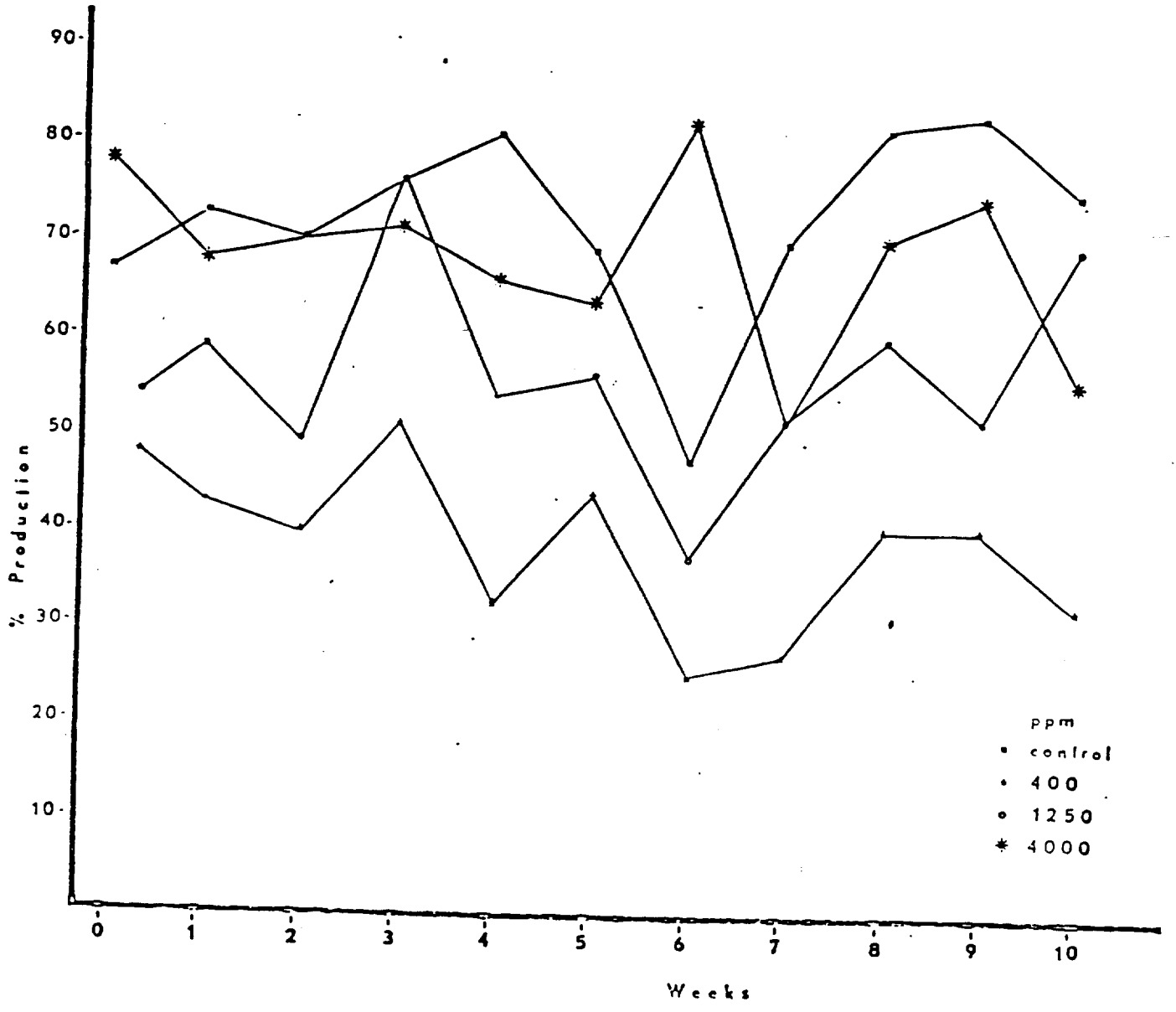

Figure 34. Effect of feeding various levels of DCPD for 28 weeks on egg production of adult Bobwhites in their first reproductive cycle. Each point represents the mean egg reproduction of fifteen females. Percents calculated from hen-day production.

of hens on 1250 ppm and 4000 ppm DCPD and the production of the control hens was found. However, production trends of all dietary groups were similar.

Analysis of incubation parameter data of Bobwhites fed DCPD-treated diets and the control diet showed no significant difference between any treated group and the control in any category. The percentages of fertile eggs were based on the number of settable eggs (total eggs laid minus cracked eggs, eggs laid by single females, and eggs used for egg shell thickness measurements). Percent hatchability, early dead, dead in shell, live in shell, pipped live, and pipped dead were based on the number of fertile eggs. Incubation parameter data are presented in Table 96.

Egg shell thickness data for Bobwhites on the DCPD study are presented in Table 97. No significant difference was found between eggshell thickness from birds fed DCPD-treated diets and birds fed a control diet. All eggs used for shell thickness measurements were included only in the calculations of total percent egg production.

14-day survival of the progeny of Bobwhites fed DCPD or control diets is plotted in Figure 35. Each point plotted is the 14-day percent survival of all progeny of all Bobwhites in a particular dietary group for a particular hatch. No significant difference between the survival of the progeny of Bobwhites fed treated feed and progeny of Bobwhites fed control feed was found.

Organ weight data for DCPD-treated and control Bobwhites are listed in Tables 98 and 99. The liver and gonad(s) weights (absolute) showed differences attributed to sex and so were separated into male and female categories. Liver weights of male Bobwhites fed 4000 ppm DCPD in the feed were significantly less than liver weights of male Bobwhites fed control feed. No other organ weights of DCPD-fed Bobwhites were significantly different from the respective organ weights of quail fed control feed.

Histopathologic examination of tissues taken from DCPD-treated and control birds showed no consistent lesions that could be attributed to the diets.

Hemoglobin values for the DCPD-study Bobwhites are presented in Table 100. There were no significant differences found between the hemoglobin values of DCPD-fed males and control males, or the hemoglobin values of DCPD-fed females and control females. However, the mean hemoglobin value of the males was significantly greater than the mean hemoglobin value of the females.

Hematocrit values are presented in Table 100. The results of the analysis of the data showed no significant difference between the hematocrit values of Bobwhites (male and female) fed DCPD diets and Bobwhites (male and female) fed control diets. However, as with the hemoglobin data analysis, the mean hematocrit of all the male Bobwhites was significantly greater than the mean hematocrit of all the female Bobwhites.

Table 96. Effect of feeding DCPD at various levels for 28 weeks on incubation parameters of Bobwhite quail eggs laid in March, April, and May, 1977

Parameter (%)	DCPD level (ppm)	Month			Combined[1]	
		March	April	May		
Cracked	0	5.06	8.21	6.21	6.49 ± 1.59	a[2]
	400	4.90	8.85	13.75	9.17 ± 4.43	a
	1250	4.14	6.41	5.00	5.18 ± 1.14	a
	4000	4.17	10.56	7.14	7.29 ± 3.20	a
Fertile	0	91.72	97.21	89.71	92.88 ± 3.88	b[2]
	400	72.79	68.93	79.41	73.71 ± 5.30	b
	1250	91.88	84.03	66.32	80.74 ± 13.09	b
	4000	82.50	91.61	84.44	86.18 ± 4.80	b
Hatched	0	80.00	75.29	81.15	78.81 ± 3.10	c[2]
	400	79.80	73.24	75.93	76.32 ± 3.30	c
	1250	78.23	61.98	84.13	74.79 ± 11.42	c
	4000	78.79	65.65	90.79	78.41 ± 12.57	c
Early dead	0	1.94	6.32	7.38	5.21 ± 2.88	d[2]
	400	6.06	8.45	16.67	10.39 ± 5.57	d
	1250	2.72	7.44	3.17	4.44 ± 2.61	d
	4000	3.79	9.17	2.63	5.20 ± 3.49	d
Dead in shell	0	4.52	3.45	3.28	3.75 ± 0.67	e[2]
	400	5.05	7.04	0.00	4.03 ± 3.63	e
	1250	4.76	4.96	1.59	3.77 ± 1.89	e
	4000	6.82	3.05	0.00	3.29 ± 3.42	e
Live in shell	0	0.65	2.30	0.82	1.26 ± 0.91	f[2]
	400	0.00	4.26	0.00	1.42 ± 2.46	f
	1250	1.36	6.61	0.00	2.66 ± 3.49	f
	4000	1.52	2.29	0.00	1.27 ± 1.17	f
Pipped live	0	12.26	12.64	7.38	10.76 ± 2.93	g[2]
	400	9.09	7.04	7.41	7.85 ± 1.09	g
	1250	12.24	18.18	11.11	13.84 ± 3.80	g
	4000	7.58	19.85	5.26	10.90 ± 7.84	g
Pipped dead	0	0.65	0.00	0.00	0.22 ± 0.37	h[2]
	400	0.00	0.00	0.00	0.00 ± 0	
	1250	0.68	0.83	0.00	0.50 ± 0.44	h
	4000	0.76	0.00	1.32	0.69 ± 0.66	h

[1] Data reported as mean ± standard deviation.

[2] Means having the same subscript are not significantly different from their respective controls (P>0.05).

Table 97. Effect of feeding DCPD at various levels for 28 weeks on shell thickness values of adult Bobwhite eggs.

DCPD level (ppm)	n	Shell thickness[1] (mm x 10-2)
0	41	21.95 ± 1.83 $_a$ [2]
400	25	21.66 ± 1.82 $_a$
1250	37	22.40 ± 2.06 $_a$
4000	37	22.25 ± 1.69 $_a$

[1] Data reported as mean ± standard deviation.

[2] Numbers with the same subscript are not significantly different from their respective control ($P>0.05$).

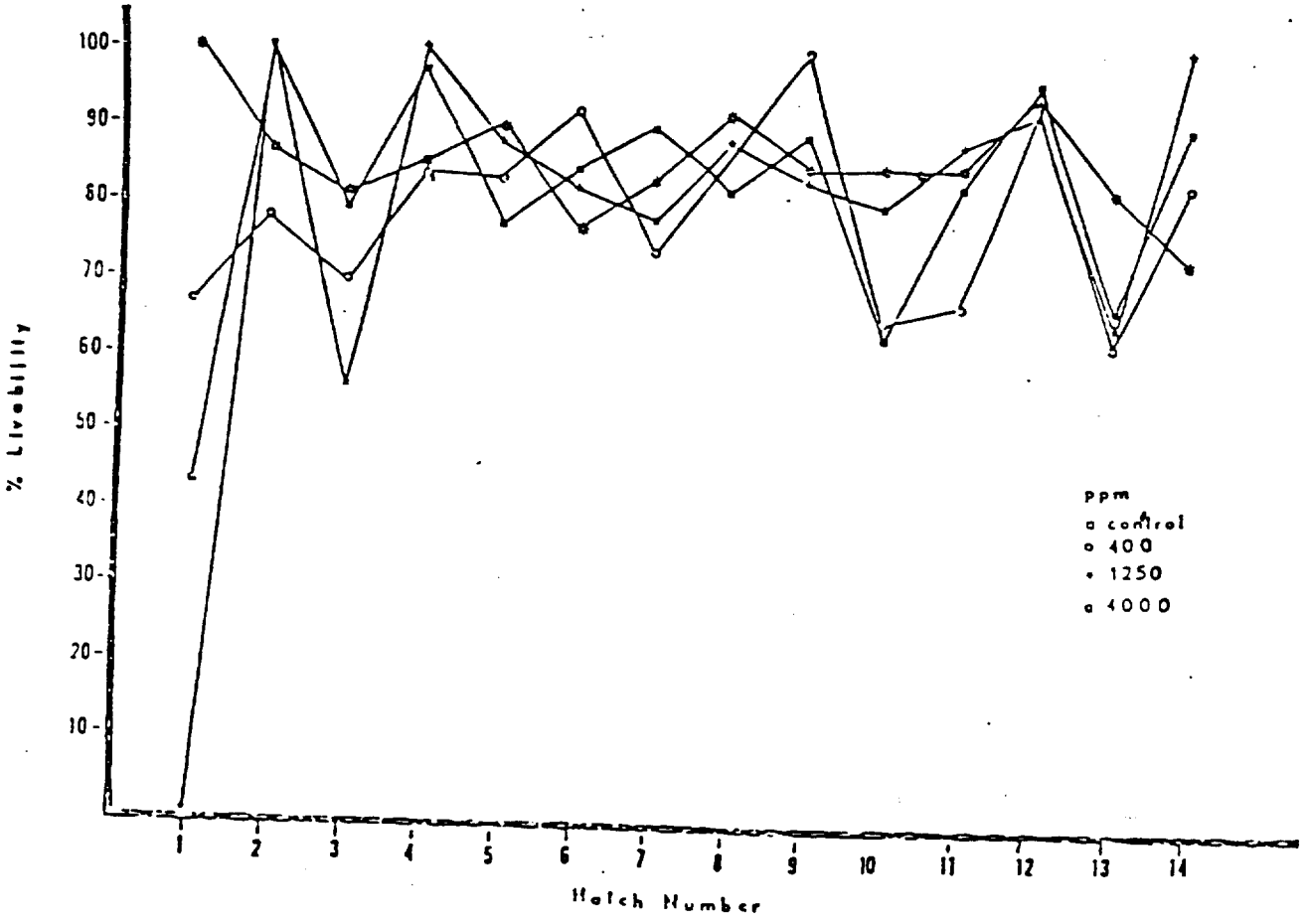

Figure 35. Percent survival of offspring of adult Bobwhites fed various levels of DCPD for 28 weeks.

Table 98. Effect of feeding DCPD at various levels for 28 weeks on liver and gonad weights of adult Bobwhites.

Sex	Organ	DCPD level (ppm)	n	Mean organ wt. (gms)	Organ weight as % of Body wt.	Organ weight as % of Brain wt.[1]
Female	Ovary	0 (control)	12	6.357	2.84	560.86 ± 174.16[2]
		400	13	7.602	3.29	655.55 ± 181.52[a]
		1250	14	6.193	2.79	560.46 ± 142.62[a]
		4000	12	7.175	3.33	622.77 ± 209.50[a]
	Liver	0	12	6.746	3.06	605.64 ± 190.72[2]
		400	13	7.211	2.88	554.35 ± 256.50[b]
		1250	14	6.689	3.03	607.07 ± 225.88[b]
		4000	12	6.263	2.71	509.79 ± 98.05[b]
Male	Testes	0	12	1.35	0.68	118.60 ± 15.20[2]
		400	14	1.14	0.57	97.74 ± 36.97[c]
		1250	13	0.98	0.49	85.71 ± 39.57[c]
		4000	13	1.28	0.65	113.34 ± 54.49[c]
	Liver	0	13	3.80	1.91	333.50 ± 65.41[2]
		400	14	4.13	2.09	355.90 ± 63.15[d]
		1250	13	3.72	1.89	323.50 ± 60.54[d]
		4000	13	3.40	1.73	263.20 ± 81.81[d]

[1] Data reported as mean ± standard deviation.

[2] Means with the same subscript are not significantly different from their respective controls ($P>0.05$).

Table 99. Effect of feeding DCPD at various levels for 28 weeks on organ weight in adult Bobwhites.

Organ	DCPD level (ppm)	n	Mean organ wt. (gms)	Organ weight as % of Body wt.	Organ weight as % of Brain wt.[1]
Spleen	0	16	.064	0.030	5.61 ± 2.53[2]
	400	15	.062	0.029	5.46 ± 3.07[a]
	1250	11	.053	0.025	4.64 ± 2.25[a]
	4000	20	.403	0.027	5.34 ± 1.65[a]
Kidneys	0	25	1.49	0.71	133.03 ± 37.60[2]
	400	27	1.33	3.30	113.61 ± 31.37[b]
	1250	27	1.50	1.08	132.82 ± 43.71[b]
	4000	25	1.44	0.70	124.93 ± 31.04[b]
Pancreas	0	25	.524	0.221	41.06 ± 12.00[2]
	400	27	.527	0.257	43.37 ± 20.51[c]
	1250	27	.467	0.224	41.47 ± 11.97[c]
	4000	25	.485	0.227	40.87 ± 12.54[c]
Proventriculus	0	25	.912	0.43	81.83 ± 18.76[2]
	400	27	.930	0.45	71.92 ± 22.55[d]
	1250	27	.971	0.47	86.37 ± 17.89[d]
	4000	25	.886	0.44	79.17 ± 16.41[d]
Gizzard	0	25	4.12	1.97	364.53 ± 61.84[2]
	400	27	3.99	1.92	342.12 ± 83.44[e]
	1250	27	4.24	2.06	376.07 ± 68.97[e]
	4000	25	4.24	2.04	369.55 ± 87.06[e]
Heart	0	25	0.96	0.49	89.24 ± 14.31[2]
	400	27	1.02	0.50	87.44 ± 19.27[f]
	1250	27	1.08	0.52	95.69 ± 22.03[f]
	4000	25	0.96	0.47	83.55 ± 18.53[f]
Brain	0	25	1.14 ±	0.09[2]	---- ----
	400	27	1.17 ±	0.11[g]	---- ----
	1250	27	1.13 ±	0.10[g]	---- ----
	4000	25	1.17 ±	0.10[g]	---- ----

[1] Data reported as mean ± standard deviation.

[2] Means with the same subscript are not significantly different from their respective controls ($P>0.05$).

Table 100. Effect of feeding DCPD at various levels for 28 weeks on Hemoglobin and Hematocrit values of adult Bobwhites.

Sex	DCPD level (ppm)	n	Hemoglobin (gm/dl.)[1]	n	Hematocrit (%)[1]
Female	0	8	9.61 ± 1.22 [2]	12	33.2 ± 5.96 [2]
	400	10	10.27 ± 0.78^{a}	13	32.9 ± 4.39^{b}
	1250	11	10.72 ± 0.91^{a}	14	35.8 ± 3.17^{b}
	4000	11	10.55 ± 1.20^{a}	12	37.0 ± 3.39^{b}
Overall		40	10.34 ± 1.073	51	34.75 ± 4.53
Male	0	10	12.28 ± 0.93	12	40.8 ± 3.38 [2]
	400	13	12.10 ± 0.96^{c}	14	40.4 ± 3.97^{d}
	1250	11	11.79 ± 0.79^{c}	13	39.1 ± 3.74^{d}
	4000	12	12.33 ± 0.85^{c}	13	43.2 ± 3.72^{d}
Overall		46	12.12 ± 0.881	52	40.87 ± 3.91

[1] Data reported as mean ± standard deviation.

[2] Means having the same subscript are not significantly different from their respective controls (P>0.05).

Data in Table 101 shows the mean corpuscular hemoglobin concentration of Bobwhites. No significant difference between the mean corpuscular hemoglobin concentrations of male and female birds fed DCPD-treated diets and their respective controls were found. Also no significant difference between the mean corpuscular hemoglobin concentration of males and females was found.

Leukocyte counts are presented in Table 102. The number of eosinophils from Bobwhites fed 1250 ppm DCPD was significantly greater than the number of eosinophils form the controls. No other significant differences between leukocyte counts of DCPD-treated Bobwhites and controls were found.

Discussion

Feed consumption was unaffected in Bobwhites fed DCPD-treated diets. During the ten-week pre-production period all groups of birds followed the same feed consumption pattern. During production, feed consumption of all groups followed a general pattern, with feed consumption steadily increasing to a peak followed by a gradual decline. This pattern is typical of normal, untreated birds during their reproductive period. Scott et al. (1969) reported that feed intake increases to accommodate for the increased energy expenditure of egg production, then decreases as egg production declines.

Pre-production body weight change of Bobwhites fed DCPD-treated diets coincided with feed consumption results and showed no treatment effects. These results are consistent with results reported for Mallards.

During the reproductive period, body weight change of Bobwhites fed DCPD-treated diets showed no treatment effects. Female birds showed a greater weight gain than male birds in all groups. This weight difference between the Bobwhite sexes is consistent with the findings of many other investigators: Stoddard (1931), Aldrich (1946), Nestler (1949), Baldini (1951), Ripley (1960), Mahmoud (1966), and Georgis (1970).

The egg production of the DCPD-fed Bobwhites showed no treatment-egg production relationship. Percent productions of birds fed the higher levels of DCPD (1250 and 4000 ppm) or control feed were near the standard value of 68.2 percent reported by Coleman(1930). Percent production of the low level group (400 ppm) was reduced but generally followed the same pattern as that of the control birds.

No effect on the incubation parameters measured was noted. The percentages of cracked eggs for each of the dietary groups and the control group were above the normal range reported in the Federal Register (1975) but did not differ significantly from the control. The percent fertility and percent hatchability of eggs produced by birds of each dietary group, including the control group, were well within the normal range reported in the Federal Register (1975).

Table 101. Effect of feeding DCPD at various levels for 28 weeks on mean corpuscular hemoglobin concentration[1] of adult Bobwhites.

DCPD level (ppm)	n females	MCHC (%)[1] females	n males	MCHC (%)[1] males	n combined	MCHC (%)[2] combined
0	10	28.93	12	30.05	22	$29.54 \pm 4.28_c$[3]
400	10	29.21	13	29.56	23	$29.41 \pm 2.12_c$
1250	12	29.51	14	28.34	26	$28.89 \pm 2.88_c$
4000	11	28.23	12	28.59	23	$28.42 \pm 1.27_c$

[1] Calculated from data in Table 69.

[2] Data reported as treatment mean ± standard deviation.

[3] Means having the same subscript are not significantly different from their respective controls ($P>0.05$).

Table 102. Effect of feeding DCPD at various levels for 28 weeks on leukocyte counts of adult Bobwhites.

Cell	DCPD level (ppm)	n	Mean[1]	Range
Basophil	0	25	2.60 ± 2.04$_a$[2]	0-8
	400	27	2.30 ± 1.64$_a$	0-5
	1250	26	2.81 ± 1.77$_a$	0-7
	4000	23	3.57 ± 2.82$_a$	0-12
Eosinophil	0	25	2.76 ± 2.57$_b$[2]	0-10
	400	27	3.33 ± 2.25$_b$	0-11
	1250	26	4.96 ± 4.50$_c$	1-14
	4000	23	3.70 ± 2.98$_b$	0-12
Heterophil	0	25	30.32 ± 14.85$_d$[2]	0-62
	400	27	27.67 ± 16.24$_d$	2-69
	1250	26	24.92 ± 15.75$_d$	5-67
	4000	23	25.83 ± 14.36$_d$	6-50
Lymphocyte	0	25	56.52 ± 18.14$_e$[2]	19-88
	400	27	57.85 ± 16.60$_e$	20-89
	1250	26	58.69 ± 20.27$_e$	2-88
	4000	23	58.91 ± 15.10$_e$	30-85
Monocyte	0	25	7.32 ± 4.68$_f$[2]	1-17
	400	27	7.52 ± 3.69$_f$	1-15
	1250	26	9.50 ± 4.60$_f$	2-17
	4000	23	8.00 ± 4.17$_f$	0-15

[1] Data reported as mean ± standard deviation

[2] Means having the same subscript are not significantly different from their respective controls ($P>0.05$).

Normal values for egg production, fertility, hatchability, and number of cracked eggs of Mallard ducks fed DCPD-treated diets were in agreement with the results of this quail study.

Two week livability of Bobwhite hatchlings from parents treated with DCPD showed no treatment effect. Percentages of chick survival of all groups, including the control group, were within the normal range reported in the Federal Register (1975).

Eggshell thichness data from DCPD-treated Bobwhites were consistent with normal values reported in the Federal Register (1975). These results were not unexpected since the Bobwhite is not susceptible to eggshell thinning (U.S. Army, 1975).

In general, the feeding of DCPD-treated diets to Bobwhites had no effect on the appearance and/or weights of their various internal organs. An exception to the preceding generality was the liver weights of the male Bobwhites fed 4000 ppm DCPD. The livers of male Bobwhites, fed 4000 ppm DCPD, weighed less than the livers of the control birds but no lesions were observed. No effect on organ weights of Mallard ducks fed DCPD-treated diets was noted.

Two blood parameters, hemoglobin concentration, and hematocrit (packed cell volume), plus the calculated mean corpuscular hemoglobin concentration, were measured in the DCPD-treated Bobwhites and found to be unaffected by treatment. The greater hemoglobin concentration and hematocrit values of the males are consistent with findings by numerous investigators. The relationship of a greater level of hemoglobin and maleness is correlated with the increased numbers of erythrocytes in the male due to testosterone.

The mean hematocrit values for male or female were within the normal ranges reported by Spiers (1978) and very near the values reported by Bond and Gilbert (1958) and Ernst et al. (1971).

Leukocyte differentials of Bobwhites fed DCPD were generally unaffected. Only the birds fed 1250 ppm DCPD showed any aberration. In the 1250 ppm DCPD group the mean number of eosinophils was significantly greater than the mean number of eosinophils in the control birds.

During the chronic study, the Bobwhites suffered a period of high mortality during a three day span in the eleventh week of the experiment. In these three days mortality was independent of DCPD dietary level. During necropsy hemorrhagic lungs were observed in all the birds that expired. No other abnormalities were observed and the cause of death could not be determined.

Mortality, other than the period of high mortality mentioned, was sporadic and not treatment related.

CONCLUSIONS

LD_{50}: Observations on mortality, feed consumption, and body weight change of Bobwhite quail show DCPD to be slightly toxic. The LD_{50} for the Bobwhite was 1010 mg/kg with a 95% confidence interval of 933.2 - 1093.1 mg/kg.

LC_{50}: The lack of treatment effect on feed consumption, mortality, and body weight gain of young Bobwhites fed DCPD-treated diets suggests that the quail could not consume enough chemical to produce sufficient mortality to calculate a LC_{50} value.

Chronic: Generally, the parameters measured in the chronic test showed no significant aberrations that could be attributed to a treatment effect. Thus, the results suggest that DCPD has little effect on Bobwhite survival and reproduction at the levels tested.